UDK 630* 159 (001)
https://doi.org./10.31298/sl.146.11-12.6

Izvorni znanstveni članci – Original scientific papers
Šumarski list, 11–12 (2022): 529–534

INFLUENCE OF BODY SIZE AND COLORATION ON THE MATE CHOICE IN STRIPED STINK BUG (*Graphosoma lineatum* L.) (HEMIPTERA: PENTATOMIDAE)

UTJECAJ VELIČINE TIJELA I OBOJENOSTI PRILIKOM ODABIRA PARTNERA KOD PRUGASTOG SMRDLJIVCA (*Graphosoma lineatum* L.) (HEMIPTERA: PENTATOMIDAE)

Linda BJEDOV[1], Marko VUCELJA[1]*, Josip MARGALETIĆ[1]

SUMMARY

Female as well as male mate choice has already been recognized in many insects. The traits responsible for the mate choice in a striped stink bug (*Graphosoma lineatum* L.) are not yet explored. This works concentrates on few possible traits responsible for mate choice in a striped stink bug. For this analysis free living copulating individuals as well as the single ones were collected. On two different locations animals were collected and their body length, pronotum broadness and coloration were measured. No differences were found in all measured traits between copulating and control individuals, showing no preferences when choosing copulation partner. Results also show no evidence of assortative mating, referring to the body length and the pronotum broadness of both sexes. All three measured traits seem not to have an influence on the mate choice of both sexes. Further experimental testing is needed to fully excluded importance of body size and coloration in mate choice of a striped stink bug.

KEY WORDS: stink bug, mate choice, body size, coloration

INTRODUCTION
UVOD

Mate choice is a common strategy in animals, where in many species females exhibit a preference for male traits and thereby potentially influence the evolution of these traits (Andersson & Simmons, 2006). This is also the case with insects where we find that different mate strategies have evolved, and that body size plays an important role in the mating success (Thornhill & Alcock, 1983). The effects of body size may also occur in both sexes (McLain & Boromisa, 1987) or only in one sex (Juliano, 1985, Hanks et al., 1996). Females show a higher fecundity with increasing body weight in most of insect taxa (Honek, 1993), so also males as well as the females do, can directly profit from choosing a bigger mate. Such preferences for a bigger sex or also assortative mating systems can be found in many taxa as smooth newts (Verrell, 1986) field crickets (Simmons, 1988), mormon crickets (Gwynne, 1981), blister beetles (Brown, 1990), leaf-cutter bees (Kim, 1997),

[1] PhD Linda Bjedov, postdoctoral, PhD Marko Vucelja, assistant professor, PhD Josip Margaletić, full professor, Department of Forest Protection and Wildlife Management, University of Zagreb, Faculty of Forestry and Wood Technology, Svetošimunska 23, 10000 Zagreb, Croatia

*Correspondence: PhD Marko Vucelja, assistant professor: mvucelja@sumfak.hr

wood frogs (Berven, 1981) and in some shield bug species, e.g. green stink bug (Capone, 1995).

Not only body size but also other traits as colour may play an important role, like in southern green stink bug, where females prefer mating with orange males rather than with the green ones (Follett et al., 2007). In a ladybird bug not only the coloration or the body size but the combination of both traits affects the male mating success where males of different body sizes obtain a mating advantage according to the colour morph (Ueno et al., 1998).

There is some data available on vibratory signals as sex attractants for the striped stink bug (*Graphosoma lineatum* L.) (Gogala 2006) but other sexual traits which could influence a mate choice are not yet known as in some other well examined Pentatomidae species. In this species bigger females and smaller males show identical coloration that mainly serves as a protection from diverse predators, signalling unpalatable prey (Veselý et al., 2006). Although this coloration shows a high similarity between the individuals as between both sexes, some colour differences are identifiable. Differences in coloration ranging from pale brownish/orange with black striation to vivid red with black striation are assumed to have cryptic role as well as aposematic and change seasonally (Sillén-Tullberg et al., 2008, Johansen et al., 2010, Gamberale-Stille et al., 2010). This colour differences as well as the body sizes of both sexes were used for this analysis. Therefore, the measurements of redness and the body size of the copulating animals were taken to provide us with some date on mate choice.

MATERIAL AND METHODS
MATERIJAL I METODE

Study organism: For this analysis only free living adults of stink bug *Graphosoma lineatum* (Linné, 1758) were used (Figure 1). The copulating individuals were collected at two different sites with similar size and vegetation. The measurements were done outdoors and the individuals were released directly afterwards on the place of finding. Additionally as control group the single found individuals of both sexes close to the copulating groups were collected.

Figure 1. Striped stink bug copulating (author: Linda Bjedov)
Slika 1. Prugasti smrdljivac u kopulaciji (autor: Linda Bjedov)

Measurements: Only the animals found on Apiaceae plants were used for measurements. The animals were collected and measured in the fields starting at 10 am. For 10 days measurements were repeated between 11th and 28th of June 2007. 65 different copulation partners, 44 control females and 53 control males were measured. The sex from all caught individuals was determined. The body length and pronotum broadness were measured with Vernier caliper. Only copulating individuals were marked with numbered white stickers. It has been calculated that body length and pronotum broadness correlate positive with each other within both sexes, excluding possible measurements mistakes (Spearman's rank correlation coefficient; female: N= 93, R= 0.654406 p< 0.000001, male: N= 107, R= 0.297051, p< 0.001). Colour of the shield was defined as red or orange. For that a paper colour scale was used with colours ranging from dark red to bright orange.

Statistics: All calculations were done with Software: StatSoft Statistica 6.0. For a correlation between the body length and pronotum broadness of both sexes Spearman's rank correlation coefficient was used. The differences in the body length and pronotum broadness between copulating and control group of both sexes were tested with Mann-Whitney-Test. Colour differences were tested with Chi^2-test, where found colour combination of copulating individuals have been tested with expected values calculated from the number of red and orange sexes within the whole population (N=201, number doesn't contain recaptured individuals).

RESULTS
REZULTATI

Average body length between individuals found copulating and the control group shows no significant difference (Figure 2). This is the case for both sexes (female: Ncop=49, Ncont=44, U=971, p=0.41; male: Ncop=54, Ncont=53, U=1393, p=0.8110). The same was found for the broadness of the pronotum (Figure 3), where both sexes of copulating group show similar pronotum broadness as the control individuals (female: Ncop=49, Ncont=44, U=949.5, p=0.3196; male: Ncop=54, Ncont=53, U=1126, p=0.0547).

The copulating individuals did not show any correlation referring to the body length or pronotum broadness (body length: N=65, R=0.0592, p=0.6392; pronotum broadness: N=65, R=0.0424, p=0.7370). Figure 4 show that the copulating partners were chosen randomly or because of some other traits and show no evidence of assortative mating.

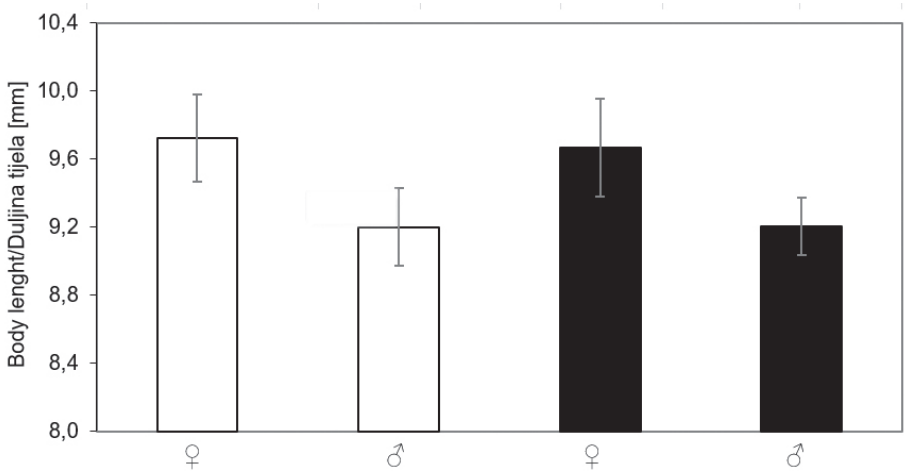

Figure 2: Average body length ± SD of animals caught copulating (white bars) and the control animals (black bars) of both sexes. The copulating bars do not contain any values from recaptured animals.
Slika 2: Prosječna duljina tijela ± SD ulovljenih kopulirajućih životinja (bijeli stupci) i kontrolnih životinja (crni stupci) za oba spola. Stupci kopulirajućih životinja ne sadrže vrijednosti ponovno ulovljenih životinja.

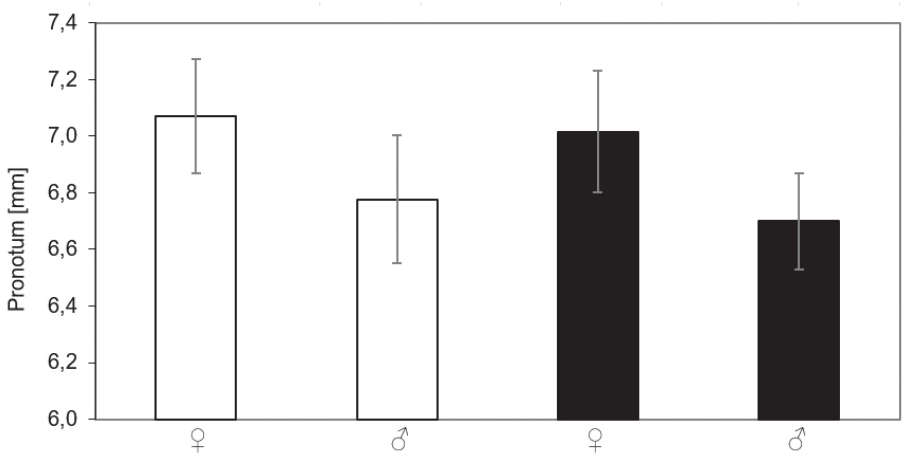

Figure 3: Average pronotum broadness ± SD of animals caught copulating (white bars) and the control animals (black bars) of both sexes. The copulating bars do not contain any values from recaptured animals.
Slika 3: Prosječna širina pronotuma ± SD ulovljenih kopulirajućih životinja (bijeli stupci) i kontrolnih životinja (crni stupci) za oba spola. Stupci kopulirajućih životinja ne sadrže vrijednosti ponovno ulovljenih životinja.

The orange colour with 63% was the dominant coloration in both sexes of copulating and control group (Table 1). No difference in amount of colour morphs between copulating and control group was found (Chi^2=1.7239, DF=3, p=0.6316).

All four possible partner combinations were found and it seems that no preference for particular colour is present. There are more orange individuals copulating with each other and other three colour combinations have similar values (Figure 5). Considering that there are more orange

Table 1. Numbers of copulating and single captured colour morphs. Table does not contain recaptured copulating animals.
Tablica 1. Broj različitih obojenosti kopulirajućih i nekopulirajućih jedinki. Podaci ne uključuju mjerenja ponovljenih kopulacija.

	Sex/Spol	Red/Crvena	Orange/Narančasta	Total/Ukupno
Copulation/Kopulacija	Female/Ženka	20	29	49
Copulation/Kopulacija	Male/Mužjak	17	38	55
Control/Kontrola	Female/Ženka	21	23	44
Control/Kontrola	Male/Mužjak	17	36	53
Total/Ukupno		75	126	201

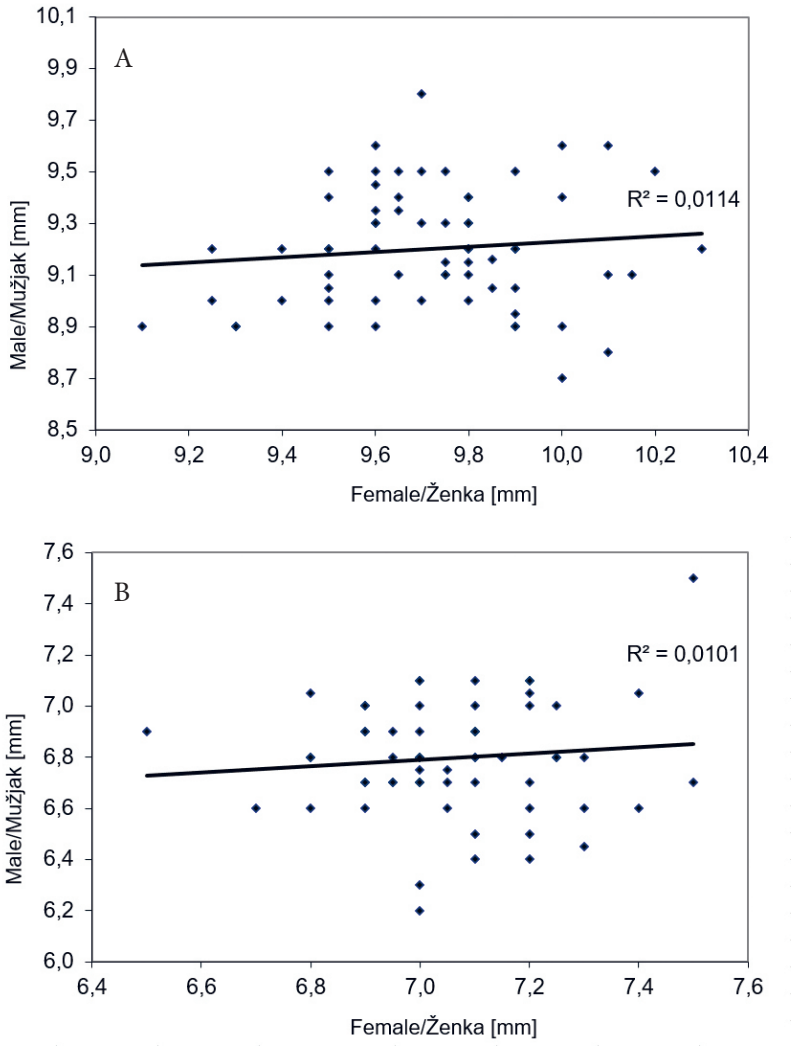

Figure 4: Correlation between the body size (A) and pronotum size (B) of copulating animals. Data does not contain points of repeated copulations (same female copulating second or more times with same male).
Slika 4: Korelacija duljine tijela (A) i širine pronotuma (B) kopulirajućih životinja. Podaci ne uključuju ponovljene kopulacije (parenja istih ženki i mužjaka dva ili više puta)

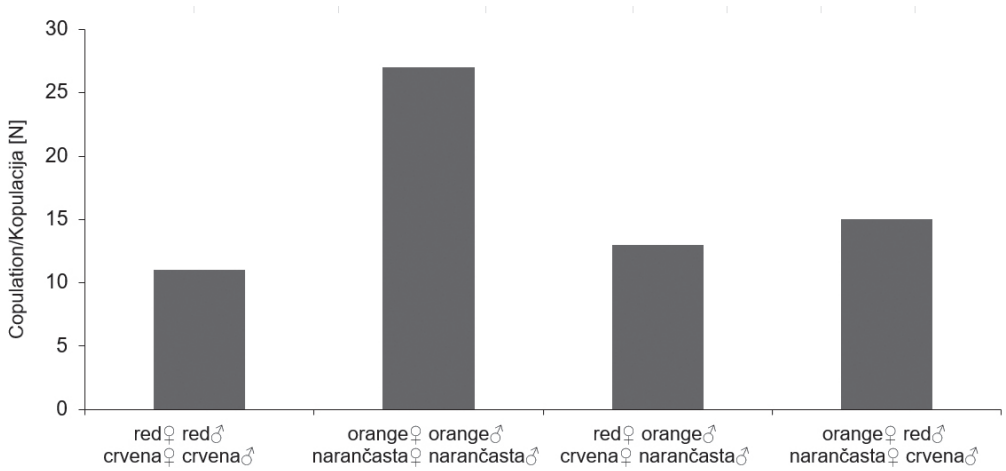

Figure 5: Absolute values of four copulation colour combination. Total number of all copulating colour combinations is 66 and contains only 1 repeated copulation.
Slika 5: Apsolutne vrijednosti četiri mogućih kombinacija obojenosti kopulirajućih jedinki. Ukupan broj svih kombinacija obojenosti kopulirajućih jedinki iznosi 66 i sadrži samo jednu ponovljenu kopulaciju.

individuals in the population, there is no evidence for preference of one colour in copulating group (Chi^2=3.8853, DF=3, p=0.2741).

DISCUSSION
RASPRAVA

This study shows that body length as well as the pronotum broadness seems not to have any effect on the mate choice in a striped stink bug. The females and males that were caught copulating do not differ in their body size from the control animals. Although body length seems to have no effect, the pronotum of copulating males appears to be slightly larger. The low p value (Ncop=54, Ncont=53, U=1126, p=0.0547) could be interpreted as a tendency for higher mating success in males with bigger pronotum. In this case an experimental approach would provide us with more information such as how strongly the environmental stimuli decrease the selective force of choosing sex for this trait.

In a green stink bug it was found that not only female preference for bigger males but also a mutual choice appears to lead to assortative mating (Capone, 1995). For our analysis no evidence for assortative mating referring to the body length or pronotum broadness could have been confirmed.

In one of Pentatomidae species (southern green stink bug, *Nezara viridula*) female preference depends on the coloration of the male (Follett et al., 2007). In our case coloration data shows that this is not the case. The copulating partner is most likely not chosen dependent on its coloration. The number of copulation combinations is proportional to the number of colour morphs in the whole population. The difference between orange and red morphs is most probably present due to some other causes and seems to have no influence on mate choice. It seems that this coloration is mostly aposematic and/or cryptic (Sillén-Tullberg et al., 2008, Johansen et al., 2010, Gamberale-Stille et al., 2010). Still the coloration shouldn't be ruled out cause aposematic role of coloration can also be used for sexual signaling (Finkbeiner et al. 2014, Rojas et.al., 2018).

This analysis has provided us with data showing that mate choice in striped stink bug considering both sexes is independent of body length, pronotum broadness and different coloration. In some Pentatomidae species there are also other traits that influence the mate choice. Such example is a green stink bug with complex mate recognition systems, where localization of a sexual partner is based on acoustical signals such as male courtship song and female calling song (Miklas et al., 2003). As in the case of red-shouldered stink bug where the pheromones are produced from the male to attract the females and not the other way around (McBrien et al., 2002), the pheromones may also play an important role in the striped stink bug. Although such traits as pheromones or vibratory signals are possible, importance of the body size and coloration in *Graphosoma lineatum* requires some further experimental testing to be excluded as having no importance in mate choice.

REFERENCES
LITERATURA

- ANDERSSON, M. & Simmons, L. W. 2006. Sexual selection and mate choice. Trends Ecol. Evol. 21: 296-302
- BERVEN, K. A. 1981. Mate choice in the wood frog, *Rana sylvatica*. Evol. 35: 707-722.
- BROWN, W. D. 1990. Size-assortative mating in the blister beetle *Lytta magister* (Coleoptera: Meloidae) is due to male and female preference for larger mates. Anim Behav. 40: 901-909
- CAPONE T, A. 1995. Mutual preference for large mates in green stink bugs, *Acrosternum hilare* (Hemiptera: Pentatomidae). Anim Behav. 49: 1335-1344
- FINKBEINER, S. D., BRISCOE, A. D., REED, R. D. 2014. Warning signals are seductive: Relative contributions of color and pattern to predator avoidance and mate attraction in Heliconius butterflies. Evolution 68-12: 3410-3420
- FOLLETT, P. A., CALVERT, F., GOLDEN, M. 2007. Genetic studies using the orange body color type of *Nezara viridula* (Hemiptera: Pentatomidae): inheritance, sperm precedence, and disassortative mating. Ann Entomol Soc Am. 100: 433-438
- GAMBERALE-STILLE, G., JOHANSEN, I. A., SILLÉN-TULLBERG, B. 2010. Change in protective coloration in the striated shieldbug *Graphosoma lineatum* (Heteroptera: Pentatomidae): predator avoidance and generalization among different life stages. Evol Ecol. 24: 423-432
- GOGALA, M., 2006: Vibratory signals produced by Heteroptera – Pentatomorpha and Cimicomorpha.- In: DROSOPOULOS, S. & CLARIDGE, M.F. (Eds.): Insect sounds and Communication: Physiology, Behaviour, Ecology and Evolution. Boca Raton, London New York: CRC Taylor and Francis, 275-296
- GWYNNE D, T. 1981. Sexual Difference Theory: Mormon Crickets Show Role Reversal in Mate Choice. Sci. 213: 779-780
- HANKS L, M., MILLAR, J. G., PAINE, T. D. 1996. Body size influences mating success of the eucalyptus longhorned borer (Coleoptera: Cerambycidae). J. Insect Behav. 9: 369-382
- HONEK, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. Oikos. 66: 483-492
- JOHANSEN, A., EXNEROVÁ, A., SVÁDOVÁ, H. K., STYS, P., GAMBERALE-STILLE, G., TULLBERG, B. 2010. Adaptive change in protective coloration in adultstriated shieldbugs *Graphosoma lineatum* (Heteroptera:Pentatomidae): test of detectability of two colour forms by avian predators. Ecol Entomol 35: 602-610
- JULIANO, S. 1985. The effects of body size on mating and reproduction in *Brachinus lateralis* (Coleoptera: Carabidae). Ecol Entomol. 10: 271-280
- KIM, J. Y. 1997. Female size and fitness in the leaf-cutter bee *Megachile apicalis*. Ecol Entom. 22: 275-282
- MCBRIEN, H. L., MILLAR, J. G., RICE, R. E., MCELFRESH, J. S., CULLEN, E., ZALOM, F. G. 2002. Sex attractant pheromone of the red-shouldered stink bug *Thyanta pallidovirens*: a

pheromone blend with multiple redundant components. J Chem Ecol. 28: 1335-1344

- MCLAIN, D. K., BOROMISA, R. D. 1987. Male choice, fighting ability, associative mating and the intensity of sexual selection in the milkweed longhorn beetle *Tetraopes tetraophthalmus* (Coleoptera: Cerambycidae). Behav Ecol Sociobiol. 20: 239-246
- MIKLAS, N., COKL, A., RENOU, M., VIRANT-DOBERLET, M. 2003. Variability of vibratory signals and mate choice selectivity in the southern green stink bug. Behav Proc 61: 131-142
- ROJAS, B., BURDFIELD-STEEL, E., DE PASQUAL, C., GORDON, S., HERNÁNDEZ, L., MAPPES, J., NOKELAINEN, O., RÖNKÄ, K., LINDSTEDT, C. 2018. Multimodal aposematic signals and their emerging role in mate attraction. Front Ecol Evol 6:93
- SILLÉN-TULLBERG, B., GAMBERALE-STILLE, G., BOHLIN, T., MERILAITA, S. 2008. Seasonal ontogenetic colour plasticity in the adult striated shieldbug *Graphosoma lineatum* (Heteroptera) and its effect on detectability. Behav Ecol Sociobiol. 62: 1389-1396
- SIMMONS, L. W. 1988. Male size, mating potential and lifetime reproductive success in the field cricket, *Gryllus bimaculatus* (De Geer). Anim Behav. 36: 372-379
- THORNHILL, R., ALCOCK, J. 1983. The evolution of insect mating systems, Harvard University Press, Cambridge, MA
- UENO, H., SATO, Y., TEUCHIDA, K. 1998. Color-associated mating success in a polymorphic ladybird beetle, *Harmonia axyridis*. Funct Ecol. 12: 757-761
- VERRELL, P. A. 1986. Male discrimination of larger, more fecund females in the smooth newt, *Triturus vulgaris*. J Herpetol. 20: 416-422
- VESELÝ, P., VESELÁ, S., FUCHS, R., ZRZAVÝ, J. 2006. Are gregarious red-black shieldbugs, *Graphosoma lineatum* (Hemiptera: Pentatomidae), really aposematic? An experimental approach. Evol Ecol Res. 8: 881-890

SAŽETAK

Odabir partnera od strane ženke, kao i mužjaka, već je prepoznat kod mnogih kukaca. Evoluirale su različite strategije odabira partnera na temelju veličine tijela i zabilježene su kod mnogih vrsta kukaca, uključujući i neke smrdibube (Hemiptera: Pentatomidae). Osim veličine tijela kod kukaca je zabilježen i utjecaj obojenosti na odabir partnera. Osobine odgovorne za odabir partnera prugastog smrdljivca (*Graphosoma lineatum* L.) nisu detaljno istražene. Ovaj se rad usredotočuje na utjecaj veličine tijela i obojenosti oba spola kod odabira partnera prugastog smrdljivca. Ukupno 10 dana su na dvije lokacije prikupljane kopulirajuće jedinke, kao i slobodne (nekopulirajuće) jedinke. Životinjama je determiniran spol, izmjerena duljina tijela, širina pronotuma i zabilježena boja. Jedinke u kopulaciji su brojevno označene u svrhu izuzimanja iz analize podatke ponovljenih parenja. Podaci uključuju ukupno 65 različitih kopulacijskih kombinacija, 44 jedinki kontrolnih ženki i 53 kontrolna mužjaka. Za sve tri mjerene osobine nisu pronađene statistički značajne razlike između kopulirajućih i kontrolnih (nekopulirajućih) jedinki, što ukazuje da ne utječu na preferencije prilikom odabira partnera. Rezultati također ne pokazuju dokaze asortativnog parenja, barem što se tiče duljine tijela i širine pronotuma. Kod oba spola čini se da sve tri mjerene osobine nemaju utjecaja na izbor partnera. Unatoč dobivenom, potrebna su daljnja ispitivanja kako bi se u potpunosti isključila važnost veličine tijela i boje u odabiru partnera prugastog smrdljivca, kao i ispitivanja vezana uz mogući utjecaj kemijskih i vibracijskih signala.

KLJUČNE RIJEČI: prugasti smrdljivac, odabir partnera, veličina tijela, obojenost