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Male–female interactions drive the (un)repeatability of copula duration in an insect

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Across the animal kingdom the duration of copulation varies enormously from a few seconds to several days. Functional explanations for this variation are largely embedded within sperm competition theory in which males modulate the duration of copula in order to optimize their fitness. However, copulation is the union of two protagonists which are likely to have separate and often conflicting reproductive interests, yet few experimental designs specifically assess the effect of male–female interactions on the duration of copulation. This can result in inexact assertions over which sex controls copulatory behaviour. Here we analyse the repeatability of copulatory behaviour in the seed beetle *Callosobruchus maculatus* to determine which sex exerts primary influence over copulation duration. In *C. maculatus*, copulation follows two distinct phases: an initial quiescent phase followed by a period of vigorous female kicking behaviour that culminates in the termination of copulation. When males or females copulated with several novel mates, copulatory behaviour was not significantly repeatable. By contrast, when males or females mated repeatedly with the same mate, copula duration was repeatable. These data suggest copulatory behaviour in *C. maculatus* to be largely the product of male–female interactions rather than the consistent, sex-specific modulation of copula duration of one protagonist in response to the phenotypic variation presented by the other protagonist.

1. Introduction

The duration of copulation varies enormously from a few seconds in the yellow fever mosquito (*Aedes aegypti*) [1] to over 10 days in the southern green stink bug (*Nezava viridula*) [2]. Functional explanations for this variation are largely embedded within sperm competition theory [3], such that males modulate the

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duration of copula in order to optimize fitness via sperm pre-emption and anti-sperm pre-emption mechanisms [4]. However, females also have a vested interest in the duration of copulation. For example, they may bias paternity in favour of particular males by varying the duration of copula in response to cues delivered by males during copulation [5,6], or they might vary the period of time spent in copula in order to optimize time allocated to other activities such as foraging, egg laying, avoiding predation and/or avoiding injury [7–9]. Thus, there are good reasons to expect females to exert some control over the duration of copulation, which may well be in conflict with the fitness optima of the male protagonist [10].

Sexual conflict over mating and copulation is well documented in the bruchid beetle *Callosobruchus maculatus*. Males have spines on their genitalia that puncture the female reproductive tract during copulation. The level of damage inflicted on females is positively related to the duration of copulation [9,11] and negatively related to female fitness ([9,11–13], although see [14]). Copulation in *C. maculatus* is characterized by two phases. In phase I, the male and female remain relatively sedentary following genital coupling, while in phase II the female vigorously kicks at her mate with her hind legs [15]. Ablation of the female's hind legs prevents females from directly kicking their mates and this results in an extended copulation duration and subsequently greater levels of genital damage [16]. This had led to the suggestion that females kick at their mates in order to reduce the duration of copulation and thus the level of harm sustained [16].

Why males prolong copula and by extension inflict greater genital damage on their mates' remains unclear. Rönn & Hotzy [13] found no evidence that the spines function as an anchor to prevent the dislodgement of copulating males by rivals, while Edvardsson & Canal [16] found no evidence that extended copulation benefited males via its effects on female re-mating propensity or male success in sperm competition (see also [11]). Hotzy *et al*. [17] reported that males with longer spines had enhanced success in sperm competition. However, they also found no evidence that spine length correlated with copulation duration, thus the relationship between spine length, copulation duration and extent of genital damage remains to be fully resolved.

That there are reproductive conflicts between the sexes in this and other species [10,18] has led a number of authors to seek which sex controls copulation duration [19–25]. However, teasing apart male and female influences over the duration of copulation is difficult. For example, pairings with large females often elicit longer copulations [26,27] which is usually interpreted as males strategically investing more ejaculate to large, fecund females (i.e. cryptic male choice) [27]. However, in comparison to small females, larger females tend to live longer [14], lay more eggs [24] and have larger sperm storage organs [28] thus, it may well be in their best interests to receive more sperm via an extended duration of copulation. One approach to circumvent these problems of interpretation is to partition variation in copulation duration between males and females that are derived from different strains (or geographical locations). In a series of intra- and inter-strain mating's involving *Drosophila mojavensis*, Krebs [26] was able to show that both male and female population origin affected the duration of copulation, with male population origin explaining five times more of the variation in copulation duration than female population origin. However, while this approach clearly partitions variation in copulation duration to male and female population origin, it does not tell us which sex exerts primary influence over copula duration within a population.

Here we examine the repeatability of copulation duration in order to identify which, if any, sex has a predominant role in determining the length of copulation in the bruchid beetle *C. maculatus*. The logic follows that if males primarily determine the duration of copulation they will modulate their behaviour according to the phenotype of the female. Thus, when the same female phenotype is presented to different males, copulation duration of the female should be repeatable (have a high intra-class correlation coefficient [29]). Should females exert primary control over copulation then the opposite holds; different females presented with the same male phenotype should have similar (repeatable) copulatory characteristics. By contrast, should copulation duration be driven by a male–female interaction, the repeatability of copulation duration when males and females are presented with different mates will be low.

2. Material and methods

The *C. maculatus* used here were originally from Niamey, Niger and at the time of the experiments had been in laboratory culture for approximately 50 generations. Stock cultures were maintained on black-eyed beans (*Vigna unguiculata*) at 27°C, approximately 35% relative humidity and a 16 L : 8 D

photoperiod. All beetles used in the experiments described below were initially virgin and between 24 and 48 h from eclosion at their first copulation. All matings and behavioural observations took place at 27°C. Under laboratory conditions about 80% of females remate within 48 h of an initial copulation so long as an adequate oviposition resource is available [30]. Female remating is also influenced by male mating status: virgin males induce longer inter-mating refractory periods in females than do non-virgin males [31].

To determine the effect of the male, the female and male–female interactions on the duration of copulation, three treatments were established: females mated repeatedly to different males, males mated repeatedly to different females, and males/females mated repeatedly to the same partner. In all treatments, focal individuals were mated four times with copulations separated by a 48 h interval, during which females laid eggs on 25 black-eyed beans. In all treatments, the mating history of the focal individual's mate was controlled such that the focal individual's 2nd, 3rd and 4th mates had previously mated once, twice and thrice, respectively. Copulation was observed under individual 50 ml Perspex pots and recorded as: (i) time from genital coupling until the female kicks at the male, and (ii) time from the onset of female kicking to genital disengagement [32]. To assess the effect of male and female size on copulation duration, male and female elytra lengths were measured under an Olympus SZH stereomicroscope linked to an image analysis workstation.

The effects of male and female identity on copula duration were assessed in a single classification ANOVA in which male (or female) identity were entered as a random independent variable and the duration of copula (and its phases) entered as the dependent variable. Intra-class correlations coefficients and their 95% confidence intervals (CIs) were calculated using the ICC package in R [33]. The duration of two of the measures of copulatory behaviour (time from genital coupling to the onset of female kicking and total duration of copula) increased with mating order (see Results and [34]). Thus, in these cases, repeatability was calculated from values normalized around the mean for each mating order, within each treatment (treatment being either copulating with the same mate or copulating with different mates).

3. Results

The duration of the start-to-kick phase of copulation increased with mating order irrespective of whether females were mated repeatedly to the same male or different males (two-way ANOVA: treatment (same or different male) $F_{1,141} = 0.24$, $p = 0.62$; mating order $F_{3,141} = 44.9$, $p < 0.0001$; figure 1a). Thus, subsequent analyses of repeatability were carried out on normalized values. When females mated to the same male on four separate occasions the duration of the start-to-kick phase was repeatable ($F_{19,55} = 2.72$, $p = 0.002$, intra-class correlation coefficient = 0.31, 95% CI 0.58–0.09). However, when females were mated to different males or males mated to different females, the duration of the start-to-kick phase was not significantly repeatable ($F_{19,54} = 1.53$, $p = 0.11$, intra-class correlation coefficient = 0.13, 95% CI 0.40−0.03 and $F_{19,54} = 0.81$, $p = 0.69$, intra-class correlation coefficient = 0.06, 95% CI 0.19−0.19, respectively). The duration of the start-to-kick phase was not related to male size, female size or the interaction between male and female size in both the same-mate and different-mate treatments ($p > 0.13$ in all cases).

Mate sequence had no effect on the duration of the kick-to-end phase of copulation (two-way ANOVA: treatment (same or different male) $F_{1,141} = 0.08$, $p = 0.77$; mating order $F_{3,141} = 0.8$, $p = 0.49$), thus intra-class correlation coefficients were calculated on the actual (i.e. non-normalized) data. When females mated to the same male on four separate occasions the duration of the kick-to-end phase of copulation was repeatable ($F_{19,55} = 2.97$, $p = 0.001$, intra-class correlation coefficient = 0.34, 95% CI 0.61–0.12). However, when females were mated to different males or males mated to different females, the duration of the kick-to-end phase was not significantly repeatable ($F_{19,54} = 0.89$, $p = 0.60$, intra-class correlation coefficient = 0.03, 95% CI 0.22−0.18 and $F_{19,54} = 1.61$, $p = 0.09$, intra-class correlation coefficient = 0.14, 95% CI 0.41−0.06, respectively). The duration of the kick-to-end phase was not related to male size, female size or their interaction in both the same-mate and different-mate treatments ($p > 0.34$ in all cases).

A similar pattern emerges from the analysis of total copulation duration. As with the duration of the first phase of copulation, total copulation duration increased with successive matings (two-way ANOVA: treatment (same or different male) $F_{1,141} = 0.39$, $p = 0.53$; mating order $F_{3,141} = 32.8$, $p < 0.0001$; figure 1b). Analyses of the normalized copulation duration values revealed the total duration of copulation to be repeatable when females mated to the same male on four separate occasions ($F_{19,55} = 4.32$, $p < 0.0001$, intra-class correlation coefficient = 0.47, 95% CI 0.70–0.24). However, when
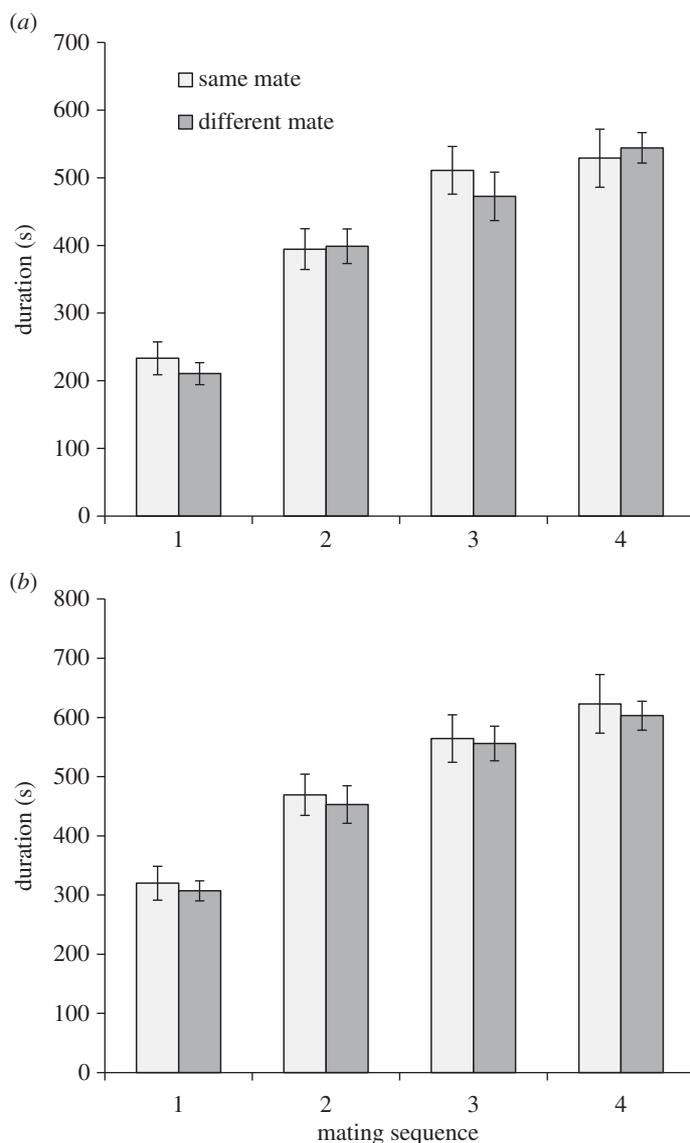
Figure 1. Mean ($\pm$s.e.) duration (seconds) of (*a*) genital coupling to the onset of female kicking and (*b*) total copulation, in relation to both mating sequence and treatment (i.e. whether copulation was with the same or different mates).

females were mated to different males the intra-class correlation coefficient was marginally non-significant ($F_{19,54} = 1.77$, $p = 0.052$, intra-class correlation coefficient $= 0.17$, 95% CI 0.45−0.03) and when males mated to different females the intra-class correlation was non-significant ($F_{19,54} = 0.52$, $p = 0.94$, intra-class correlation coefficient $= -0.15$, 95% CI 0.05−0.25). Total copulation duration was not related to male size, female size or their interaction in both the same-mate and different-mate treatments ($p > 0.14$ in all cases).

4. Discussion

Here we have shown copulation duration in the seed beetle *C. maculatus* to be largely the product of a male–female interaction. Neither males nor females were consistent in their copulatory behaviour when paired with different mates at each copulation. This lack of repeatability could arise from males and females adjusting their behaviours in response to cues derived from dynamic internal states and external stimuli such that each copulatory event represents a unique set of circumstances to both interacting parties. For a male, salient external stimuli might include female status (virgin or not), female fecundity (size), the likelihood of finding another mate or the likelihood of facing sperm competition [4,27], while females might vary the duration of copulation in response to male quality [5,6] and/or time constraints

associated with other activities, such as finding suitable oviposition sites. Some of these external stimuli could be conveyed via the hydrocarbon profiles of both males and females as these are known to vary ontogenetically and in response to social cues [35]. They have also been shown to affect the duration of copula in *Drosophila melanogaster* [36]. However, apart from male and female quality, external factors were held constant across the same-mate/different-mate treatments, plus there was no evidence that absolute or relative mate size affected the duration of copula. Thus, had males strategically invested more ejaculate in larger females, we would have expected to observe consistently longer copulations when males were paired with large females. Low levels of copulation duration repeatability have been previously reported for the Queensland fruit fly (*Bactrocera tryoni*) when males copulated with different mates ($r < 0.1$; [23,37]).

That copulation duration is a product of a male–female interaction also makes sense from a functional perspective as under a polyandrous mating strategy the two sexes are likely to have different fitness optima associated with time spent in copula [6,10] and are thus likely to evolve traits that enable them to shape the duration of copula towards their own sex-specific optima. Which sex wins this conflict appears to depend on the value of winning and power (the relative contest costs of the two sexes [38]). Males typically have higher values of winning [39] but power may be loaded in favour of the female (especially in invertebrates where females are typically larger than males). Thus, we expect copulation duration to be influenced by male and female traits. Despite this, a number of studies point to male or female control over the duration of copulation. For example, Crickmore & Vosshall [40] show that copulation duration in *D. melanogaster* is under the control of the male (see also [41]), which is primarily driven by the interplay between GABAergic interneurons of the male abdominal ganglion and dopaminergic neurons of the ventral nerve cord. By contrast, females appear to exert control over copulation duration in *Drosophila montana*. In this species copulation appears to bear many similarities to that observed in *C. maculatus* in that the initial stages of copulation appear harmonious while the latter stages are characterized by vigorous kicking at the mounted male by the female. Males copulating with dead females (i.e. no female kicking) engaged in copula that was substantially longer than those copulating with normal females [42]. Similar experiments in the Queensland fruit fly (*B. tryoni*) also point to female control (or at least influence) over copulation duration [23] as do the results of leg ablation experiments in female *C. maculatus* [16].

The low repeatability of copulation duration would indicate low heritability [43]. Despite this, several studies report heritable variation in the duration of copulation in insects [44–46] including *C. maculatus* [12,20,47]. Low repeatability and significant heritable variation could result from different studies using different populations (heritability is a population specific measure) or different experimental approaches. For example, Savalli & Fox [20] and Brown *et al.* [47] estimated the heritability of copulation duration based on matings between virgin adults, whereas here we estimated repeatability when individuals were either virgin (1st mating) or non-virgin (subsequent matings). Similar inconsistencies exist in the *Drosophila* literature: Gromko [44] found copulation duration to be heritable in *D. melanogaster* while Taylor *et al.* [48] found no evidence for heritable variation in this behaviour and suggested this was owing to the inherent phenotypic and genetic variability of the females used to assay the copulation duration of focal males.

That copulation duration increases with copulatory sequence (see also [34]) suggests copulation duration is negatively related to the size of the ejaculate transferred in this species. This is based on the well-established observation that *C. maculatus* males transfer smaller ejaculates [49] and fewer sperm [31] at each successive mating. Similar results can be inferred from the studies of Ofuya [50] and Fox *et al.* [49] which show older males to both spend longer in copula and transfer smaller ejaculates than younger males, and Vasudeva *et al.* [51] who found males exposed to thermal stress during development transferred fewer sperm but spent longer in copula than males reared under optimal conditions. Taken together, these studies indicate copulation duration to be negatively related to male quality in *C. maculatus* and could account for the negative phenotypic and genotypic association between copulation duration and longevity found in this species [47]. However, the relationship between copulation duration and ejaculate size is clearly a complex one as Edvardson & Canal [16] and van Leishout *et al.* [52] report ejaculate weight to increase with increasing copulation duration, while Savalli & Fox [20] and Brown *et al.* [47] found no relationship. The upshot of these conflicting results is that caution should be exercised when interpreting the adaptive value of extended copulation without detailed knowledge of its relationship with sperm and ejaculate transfer.

References

1. Spielman A. 1964 The mechanics of copulation in *Aedes aegypti*. *Biol. Bull.* **127**, 324–344. (doi:10.2307/1539230)

2. Mitchell WC, Mau RFL. 1969 Sexual activity and longevity of the southern green stink bug, *Nezara viridula*. *Ann. Entomol. Soc. Am.* **62**, 1246–1247. (doi:10.1093/aesa/62.6.1246)

3. Parker GA. 1970 Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**, 525–567. (doi:10.1111/j.1469-185X.1970.tb01176.x)

4. Simmons LW. 2001 *Sperm competition and its evolutionary consequences in the insects*. Princeton, NJ: Princeton University Press.

5. Thornhill R. 1976 Sexual selection and nuptial feeding behaviour in *Bittacus apicalis* (Insecta: Mecoptera). *Am. Nat.* **110**, 529–548. (doi:10.1086/283089)

6. Eberhard WG. 1996 *Female control: sexual selection by cryptic female choice*. Princeton, NJ: Princeton University Press.

7. Rowe L. 1994 The costs of mating and mate choice in water striders. *Anim. Behav.* **48**, 1049–1056. (doi:10.1006/anbe.1994.1338)

8. Krupa JJ, Sih A. 1998 Fishing spiders, green sunfish and a stream water-strider: male-female conflict and prey responses to single versus multiple predator environment. *Oecologia* **117**, 258–265. (doi:10.1007/s004420050656)

9. Crudgington HS, Siva-Jothy MT. 2000 Genital damage, kicking and early death. *Nature* **407**, 855–856. (doi:10.1038/35038154)

10. Arnqvist G, Rowe L. 2005 *Sexual conflict*. Princeton, NJ: Princeton University Press.

11. Edvardsson M, Tregenza T. 2005 Why do male *Callosobruchus maculatus* harm their mates? *Behav. Ecol.* **16**, 788–793. (doi:10.1093/beheco/ari055)

12. Gay L, Hosken DJ, Eady PE, Vasudev R, Tregenza T. 2011 The evolution of harm—effect of sexual conflicts and population size. *Evolution* **65**, 725–737. (doi:10.1111/j.1558-5646.2010.01181.x)

13. Rönn JL, Hotzy C. 2012 Do longer genital spines in male seed beetles function as better anchors during mating? *Anim. Behav.* **83**, 75–79. (doi:10.1016/j.anbehav.2011.10.007)

14. Eady PE, Hamilton L, Lyons RE. 2007 Copulation, genital damage and early death in *Callosobruchus maculatus*. *Proc. R. Soc. B* **274**, 247–252. (doi:10.1098/rspb.2006.3710)

15. Qi YT, Burkholder WE. 1982 Sex pheromone biology and the behaviour of the cowpea weevil *Callosobruchus maculatus* (Coleoptera: Bruchidae). *J. Chem. Biol.* **8**, 527–534.

16. Edvardsson M, Canal D. 2006 The effects of copulation duration in the bruchid beetle *Callosobruchus maculatus*. *Behav. Ecol.* **17**, 430–434. (doi:10.1093/beheco/arj045)

17. Hotzy C, Polak M, Rönn JL, Arnqvist G. 2012 Phenotypic engineering unveils the function of genital morphology. *Curr. Biol.* **22**, 2258–2261. (doi:10.1016/j.cub.2012.10.009)

18. Parker GA. 1979 Sexual selection and sexual conflict. In *Sexual selection and reproductive competition in insects* (eds MS Blum, NS Blum), pp. 123–166. New York, NY: Academic Press.

19. Arnqvist G, Rowe L. 1995 Sexual conflict and arms races between the sexes: a morphological adaptation for control of mating in a female insect. *Proc. R. Soc. Lond. B* **261**, 123–127. (doi:10.1098/rspb.1995.0126)

20. Savalli UM, Fox CW. 1998 Genetic variation in parental investment in a seed beetle. *Anim. Behav.* **56**, 953–961. (doi:10.1006/anbe.1998.0853)

21. Baer B. 2003 Bumblebees as model organisms to study male sexual selection in social insects. *Behav. Ecol. Sociobiol.* **54**, 521–533. (doi:10.1007/s00265-003-0673-5)

22. Wilder SM, Rypstra AL. 2007 Male control of copulation duration in a wolf spider (Araneae, Lycosidae). *Behaviour* **144**, 471–484. (doi:10.1163/156853907780756012)

23. Pérez-Staples D, Weldon CW, Radhakrishnan P, Prenter J, Taylor PW. 2010 Control of copula duration and sperm storage by Queensland fruit flies. *J. Insect Physiol.* **56**, 1755–1762. (doi:10.1016/j.jinsphys.2010.07.005)

24. Barbosa F. 2011 Copulation duration in the soldier fly: the roles of cryptic male choice and sperm competition risk. *Behav. Ecol.* **22**, 1332–1336. (doi:10.1093/beheco/arr137)

25. Wilson CJ, Tompkins JL. 2014 Countering counteradaptations: males hijack control of female kicking behaviour. *Behav. Ecol.* **25**, 470–476. (doi:10.1093/beheco/aru022)

26. Krebs RA. 1991 Function and genetics of long versus short copulations in the cactophilic fruit fly, *Drosophila mojavensis* (Diptera: Drosophilidae). *J. Insect Behav.* **4**, 221–233. (doi:10.1007/BF01054614)

27. Kelly CD, Jennions MD. 2011 Sexual selection and sperm quantity: meta-analyses of strategic ejaculation. *Biol. Rev. Camb. Philos. Soc.* **86**, 863–884. (doi:10.1111/j.1469-185X.2011.00175.x)

28. Parker GA, Simmons LW, Stockley P, McChristy DM, Charnov EL. 1999 Optimal copula duration in yellow dung flies: effects of female size and egg content. *Anim. Behav.* **57**, 795–805. (doi:10.1006/anbe.1998.1034)

29. Lessells CM, Boag PT. 1987 Unrepeatable repeatabilities: a common mistake. *Auk* **104**, 116–121. (doi:10.2307/4087240)

30. Eady PE, Rugman-Jones PF, Brown DV. 2003 Prior oviposition, female receptivity and last-male sperm precedence in *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Anim. Behav.* **67**, 559–565. (doi:10.1016/j.anbehav.2003.07.003)

31. Eady PE. 1995 Why do male *Callosobruchus maculatus* beetles inseminate so many sperm? *Behav. Ecol. Sociobiol.* **36**, 25–32. (doi:10.1007/BF00175725)

32. Eady PE. 1994 Intraspecific variation in sperm precedence in the bruchid beetle *Callosobruchus maculatus*. *Ecol. Entomol.* **19**, 11–16. (doi:10.1111/j.1365-2311.1994.tb00384.x)

33. R Developmental Core Team. 2012 *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See http://www.R-project.org.

34. Paukku S, Kotiaho JS. 2005 Cost of reproduction in *Callosobruchus maculatus*: effects of mating on male longevity and the effect of male mating status on female longevity. *J. Insect Physiol.* **51**, 1220–1226. (doi:10.1016/j.jinsphys.2005.06.012)

35. Ingleby FC. 2015 Insect cuticular hydrocarbons as dynamic traits in sexual communication. *Insects* **6**, 732–742. (doi:10.3390/insects6030732)

36. Marcillac F, Ferveur JF. 2004 A set of female pheromones affects reproduction before, during and after mating in *Drosophila*. *J. Exp. Biol.* **207**, 3927–3933. (doi:10.1242/jeb.01236)

37. Radhakrishnan P, Péres-Staples D, Weldon CW, Taylor PW. 2009 Multiple mating and sperm depletion in male Queensland fruit flies: effects on female remating behaviour. *Anim. Behav.* **78**, 839–846. (doi:10.1016/j.anbehav.2009.07.002)

38. Parker GA, Partridge L. 1998 Sexual conflict and speciation. *Phil. Trans. R. Soc. Lond. B* **353**, 261–274. (doi:10.1098/rstb.1998.0208)

39. Trivers RL. 1972 Parental investment and sexual selection. In *Sexual selection and the descent of man, 1871–1971* (ed. B Campbell), pp. 136–172. Chicago, IL: Aldine-Atherton.

40. Crickmore MA, Vosshall LB. 2013 Opposing dopaminergic and GABAergic neurons control the duration and persistence of copulation in *Drosophila*. *Cell* **155**, 881–893. (doi:10.1016/j.cell.2013.09.055)

41. Beaver LM, Giebultowicz JM. 2004 Regulation of copulation duration by *period* and *timeless* in *Drosophila melanogaster*. *Curr. Biol.* **14**, 1492–1497. (doi:10.1016/j.cub.2004.08.022)

42. Mazzi D, Kesäniemi J, Hoikkala A, Klappert K. 2009 Sexual conflict over the duration of copulation in *Drosophila montana*: why is longer better? *BMC Evol. Biol.* **9**, 132. (doi:10.1186/1471-2148–9–132)

43. Boake CRB. 1989 Repeatability: its role in evolutionary studies of mating behaviour. *Evol. Ecol.* **3**, 173–182. (doi:10.1007/BF02270919)

44. Gromko MH. 1987 Genetic constraint on the evolution of courtship behaviour in *Drosophila melanogaster*. *Heredity* **62**, 251–255. (doi:10.1038/hdy.1989.35)

45. Gromko MH, Briot A, Jensen SC, Fukui HH. 1991 Selection on copulation duration in *Drosophila melanogaster*: predictability of direct response

versus unpredictability of correlated response. *Evolution* **45**, 69–81. (doi:10.2307/2409483)

46. Mühlhäuser C, Blanckenhorn WU, Ward PI. 1996 The genetic component of copulation duration in the yellow dung fly. *Anim. Behav.* **51**, 1401–1407. (doi:10.1006/anbe.1996.0143)

47. Brown EA, Gay L, Vasudev R, Tregenza T, Eady PE, Hosken DJ. 2009 Negative phenotypic and genetic associations between copulation duration and longevity in male seed beetles. *Heredity* **103**, 340–345. (doi:10.1038/hdy.2009.80)

48. Taylor ML, Evans JP, Garcia-Gonzales F. 2013 No evidence of heritability of male mating latency or copulation duration across social environments in *Drosophila melanogaster*. *PLoS ONE* **8**, e77347. (doi:10.137/journal.pone.0077347)

49. Fox CW, Hickman DL, Raleigh EL, Mousseau TA. 1995 Paternal investment in a seed beetle (Coleoptera: Burchidae): influence of male size, age, and mating history. *Ann. Entomol. Soc. Am.* **88**, 100–103. (doi:10.1093/aesa/88.1.100)

50. Ofuya TI. 1995 Multiple mating and its consequences in males of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *J. Stor. Prod. Res.* **31**, 71–75. (doi:10.1016/0022-474X(94)00031-N)

51. Vasudeva R, Deeming DCD, Eady PE. 2014 Developmental temperature affects the expression of ejaculatory traits and the outcome of sperm competition *in Callosobruchus maculatus*. *J. Evol. Biol.* **27**, 1811–1818. (doi:10.1111/jeb.12431)

52. Van Lieshout E, McManara KB, Simmons LW. 2014 Why do female *Callosobruchus maculatus* kick their mates? *PLoS ONE* **9**, e95747. (doi:10.1371/journal.pone.0095747)

53. Eady PE, Brown DV. 2017 Data from: Male–female interactions drive the (un)repeatability of copula duration in an insect. Dryad Digital Repository. (http://dx.doi.org/10.5061/dryad.b0h4h)