



Female mating preferences for outbred versus inbred males are conditional upon the female's own inbreeding status



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Inbreeding occurs when relatives mate with each other, and it often has detrimental effects for the fitness of any resulting offspring. It is an important issue in ecology and evolutionary biology with profound implications for genetic variation and the evolution of mating systems and reproductive strategies. For example, inbreeding may shape mate choice through the avoidance of outbred, related individuals to prevent inbreeding, or through the avoidance of inbred, unrelated individuals that have been produced through inbreeding. Although the former has been studied extensively, little is known about mating preferences based on the inbreeding status of potential partners. It is also unclear whether these mating preferences are influenced by the inbreeding status of the choosing sex. Here, we examined female mating preferences for outbred versus inbred males using dichotomous choice tests in the burying beetle *Nicrophorus vespilloides*. We found that these mating preferences were conditional upon the female's own inbreeding status: inbred females preferentially mated with outbred males, whereas outbred females did not show such a preference. Our findings suggest that inbred males suffer reduced mating success only when interacting with inbred females. In species where this is the case, the fitness costs of inbreeding with respect to male mating success will therefore depend on the frequency of inbred females relative to outbred females, which depends on the rate of inbreeding in the population.

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Inbreeding refers to the mating between close relatives and is often associated with a reduction in the fitness of any resulting offspring, known as inbreeding depression (Charlesworth & Charlesworth, 1987). These fitness costs are due to a general loss of heterozygosity, which increases the likelihood that recessive, deleterious alleles are expressed (Charlesworth & Charlesworth, 1987). Given its detrimental effects for the fitness of inbred offspring, inbreeding may influence mate choice, which is the outcome of interactions between males and females, with females usually being the choosing sex and males the competing sex (Andersson, 1994).

Inbreeding can affect mating patterns at two distinct levels. First, the costs of inbreeding may lead to mating preferences for unrelated over related individuals. Active mate choice is a key mechanism for inbreeding avoidance, whereby individuals avoid mating with relatives to reduce the risk of producing inbred

offspring (Frommen & Bakker, 2006; Gerlach & Lysiak, 2006; Hansson et al., 2007). Second, females might avoid mating with inbred, unrelated partners if outbred, unrelated partners are of higher quality (Ilmonen, Stundner, Thoss, & Penn, 2009). Hence, inbreeding may shape mate choice through the avoidance of related individuals to prevent inbreeding and/or through the avoidance of low-quality individuals produced by inbreeding.

Inbreeding avoidance by active mate choice has been studied extensively across a wide range of taxa (Pusey & Wolf, 1996; Szulkin, Stopher, Pemberton, & Reid, 2013; Tregenza & Wedell, 2000). This work has focused on when and why animals may avoid, tolerate or in some cases prefer to mate with their relatives (Kokko & Ots, 2006; Szulkin et al., 2013). Relatively little is known about whether and when inbred individuals might be less preferred as potential mates, although a growing number of empirical studies in mammals, birds, fishes and insects have shown that outbred partners are typically preferred over inbred ones (Ala-Honkola et al., 2015; Bolund, Martin, Kempnaers, & Forstmeier, 2010; Ilmonen et al., 2009; McKee, Newton, & Carter, 2014; Okada, Blount, Sharma, Snook, & Hosken, 2011; Pölkki, Krams, Kangassalo, & Rantala, 2012; Zajitschek & Brooks, 2010; but see also Drayton, Milner, Hunt, & Jennions, 2010; Michalczyk, Martin,

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Millard, Emerson, & Gage, 2010). Theoretical work suggests that these preferences for outbred males are unlikely to be driven by indirect (genetic) benefits, because homozygosity is not heritable (Lehmann, Keller, & Kokko, 2007; Reinhold, 2002; but see Neff & Pitcher, 2008; Nietlisbach, Keller, & Postma, 2016). A more likely explanation is that inbreeding reduces overall male quality and condition such that females gain fewer direct benefits from mating with an inbred male (Fox, Xu, Wallin, & Curtis, 2012). Direct benefits that may be affected by inbreeding include sperm number and quality, nuptial gift size and parental care ability (Fox et al., 2012).

Traditionally, studies on female mate choice have focused only on the inbreeding status of males, giving outbred females a choice between outbred and inbred males. Nevertheless, the females' own inbreeding status might also influence their mating preferences. If low-quality females cannot afford the costs of being choosy (Burley & Foster, 2006; Cotton, Small, & Pomiankowski, 2006; Hunt, Brooks, & Jennions, 2005; Ilmonen et al., 2009; McKee et al., 2014), we might expect a stronger mating bias towards outbred males by outbred (high-quality) females than by inbred (low-quality) females. On the other hand, if the benefits gained from being choosy are inversely related to female quality, inbred females should have a stronger preference for outbred males to compensate for their own shortcomings (Ilmonen et al., 2009). It is important to better understand how inbreeding affects female choosiness since this may have important implications for sexual selection dynamics in inbred populations.

In this study, we used the burying beetle *Nicrophorus vespilloides*, to test whether females preferentially mate with outbred over inbred males and whether female choosiness is influenced by the female's own inbreeding status. Matthey and Smiseth (2015a) found no evidence for inbreeding avoidance in this species despite severe inbreeding depression in the offspring (Matthey, Strutt, & Smiseth, 2013; Pilakouta, Jamieson, Moorad, & Smiseth, 2015; Pilakouta, Sieber, Smiseth, 2016; Pilakouta & Smiseth, 2016) and heavy investment by both sexes in parental care (Pilakouta, Richardson, & Smiseth, 2015; Smiseth, Dawson, Varley, & Moore, 2005; Smiseth & Moore, 2004). Nevertheless, it is possible that females exhibit mating preferences based on the inbreeding status rather than the relatedness of potential partners. To test this, we conducted dichotomous choice tests during which we recorded the copulation rate of an outbred or inbred female presented with two potential mates, one outbred and one inbred. We predicted that females would avoid mating with inbred males, because they are low-quality mates (Matthey et al., 2013). We also expected that outbred and inbred females would differ in their choosiness, but we did not have an a priori prediction about the direction of this effect.

METHODS

Beetle Husbandry

We used virgin beetles from an outbred laboratory population maintained at the University of Edinburgh. The beetles used in this study comprised second- and third-generation beetles from lines originally collected in Edinburgh, U.K. They were housed individually in transparent plastic containers (12 × 8 cm and 2 cm high) filled with moist soil and kept at 22 °C and a 16:8 h light:dark cycle. All nonbreeding adults were fed small pieces of raw organic beef twice a week.

Generating Outbred and Inbred Beetles

In the first part of our experiment, we generated outbred and inbred males and females for use in the mate choice trials. To produce outbred individuals, we paired outbred beetles ($N = 25$)

that had no common ancestors for at least two generations. To produce inbred individuals, we paired outbred beetles ($N = 25$) that were full siblings. Each pair ($N = 50$) was placed in a transparent plastic container (17 × 12 cm and 6 cm high) filled with 1 cm of moist soil. Burying beetles use carcasses of small vertebrates as a breeding resource, so we provided each of these pairs with a freshly thawed mouse carcass (Livefoods Direct Ltd, Sheffield, U.K.). We did not disturb them until the larvae started dispersing from the carcass, which occurs approximately 5 days after hatching. At the dispersal stage, we placed five larvae from each brood into individual containers (12 × 8 cm and 2 cm high) filled with moist soil. The inbred and outbred offspring eclosed as adults about 20 days later, at which point they were sexed based on differences in the terminal segments of the abdomen (Trumbo, 1996). We only used one female and two males from each family. We also recorded the body size of all individuals by measuring their pronotum width using a digital calliper with a precision of 0.01 mm (Bartlett & Ashworth, 1988).

Dichotomous Choice Tests

In this species, adult beetles become sexually mature around 10 days after eclosion. For our mate choice trials, we only used virgin beetles aged between 10 and 20 days after eclosion to minimize variation in male and female age and to prevent variation due to previous mating experience. Each trial consisted of a single outbred or inbred female that was given a choice between an outbred and an inbred male. This design simulates a situation where a female encounters multiple males on a carcass in the wild (i.e. simultaneous mate choice). In half of the trials, we used an outbred female ($N = 15$) and in the other half we used an inbred female ($N = 15$). The two males used in each trial were size-matched based on their pronotum width (difference <0.10 mm) to exclude differences in female mating preferences due to male size. We always used unrelated individuals in each trial.

Mate choice trials took place in a transparent container (17 × 12 cm and 6 cm high) filled with 0.5 cm of moist soil and a freshly thawed mouse carcass (Livefoods Direct Ltd, Sheffield, U.K.) of a standardized size (27–30 g). We first tethered each male by tying one end of a piece of dental floss around the male's pronotum and taping the other end to the side of the box. The two males were tethered to opposite sides of the box to prevent competition, which otherwise would restrict the female's ability to choose between them (Otronen, 1988). We tied the dental floss such that there was about 3 cm of give to ensure that we did not limit the males' ability to mount and mate with the female (Matthey & Smiseth, 2015a). Both males could reach the carcass, which was placed in the middle of the box, but they could not come in direct contact with each other. We alternated between trials whether the outbred or inbred male was tethered on the side close to the front versus the back of the carcass (Matthey & Smiseth, 2015a).

At the start of the trial, we placed the female at the centre of the carcass such that she was equidistant from the two males. We recorded the time when the female first came into contact with the outbred and the inbred male and the number of copulations she had with each male over the next 45 min. Successful copulations occurred when the male inserted his aedeagus (intromittent organ) into the female's vagina (House et al., 2008). Given that each copulation typically lasts about 90 s and females do not have a refractory period (House et al., 2008), it was possible for females to mate repeatedly with the same male or both males. All outbred and inbred females mated at least once over the course of the 45 min mate choice trial. All trials ($N = 30$) were included in the analyses described below.

Data Analysis

A female might show a preference for the outbred male either by only mating with that male or by mating with the outbred male more times than she mates with the inbred male. To examine the former scenario, we used a generalized linear model (GLM) where the response variable indicated whether a female mated with only one male or both males during the dichotomous choice test. Our two explanatory variables were female inbreeding status (outbred or inbred) and female pronotum width. This model was fitted using a binomial error distribution with a complementary log-log link function. To examine the latter scenario, we first tested for a negative correlation between the number of times the female copulated with the outbred and inbred male in each trial, which would indicate that mating with one male reduced the likelihood of mating with the other male. After confirming the absence of such a correlation (Spearman rank test: $\rho = 0.063$, $P = 0.74$), we tested whether females copulated more frequently with the outbred or the inbred male, using a generalized linear mixed model (GLMM) with a Poisson error distribution ('glmer' function in the 'lme4' package). The starting model included the following factors: male inbreeding status (outbred or inbred), female inbreeding status (outbred or inbred), female pronotum width, the male's position relative to the carcass (front or back), and whether that male was the first the female interacted with (yes or no). Female identity was added as a random effect to account for the nonindependence between the observations on the two males in the same trial. Decisions about which variables to include in the final model were based on AIC model selection criteria to obtain the minimal adequate model. After model simplification, our final model included the following factors: male inbreeding status, female inbreeding status and the interaction between male and female inbreeding status. Statistical results for factors dropped from the final model (i.e. female pronotum width, the male's position relative to the carcass and whether he was the first male the female interacted with) are the values from the full model prior to being removed. This model was fitted using maximum likelihood methods. Lastly, we used a Mann-Whitney U test to compare the total number of copulations by outbred and inbred females, as a measure of female mating activity or eagerness to mate. We used R version 3.3.1 (R Core Team, 2013) for all analyses.

Ethical Note

Our study adheres to the ASAB/ABS Guidelines for the Use of Animals in Research, the legal requirements of the U.K. and all institutional guidelines at The University of Edinburgh. None of the procedures used in this study had the potential to cause pain or distress to the beetles.

RESULTS

We found that female mating preferences for inbred versus outbred males were conditional upon the female's own inbreeding status (Fig. 1). Inbred females copulated more with outbred males, while outbred females showed no preference between outbred and inbred males (GLMM: male inbreeding status: LR $\chi^2_1 = 5.47$, $P = 0.02$; female inbreeding status: LR $\chi^2_1 = 0.87$, $P = 0.35$; interaction: LR $\chi^2_1 = 9.01$, $P < 0.01$). These mating preferences were not influenced by female pronotum width (GLMM: LR $\chi^2_1 = 0.15$, $P = 0.70$), whether the male was tethered to the side closest to the front or back of the carcass (GLMM: LR $\chi^2_1 = 0.54$, $P = 0.46$) or which male the female interacted with first (GLMM: LR $\chi^2_1 = 0.34$, $P = 0.56$). Inbred females were also less likely to mate with both males during the dichotomous choice test (GLM: $\chi^2_1 = 0.432$,

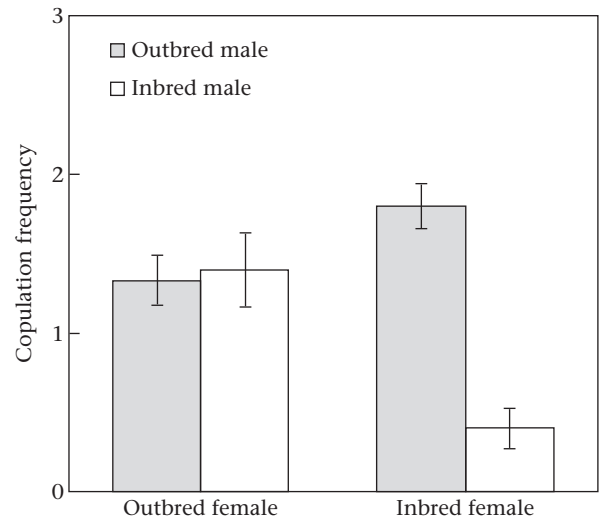


Figure 1. Mean \pm SE number of times an outbred or inbred female mated with the outbred male (grey) and the inbred male (white) during a 45 min mate choice trial.

$P = 0.038$; Fig. 2). Female pronotum width did not influence the likelihood of mating with both males (GLM: $\chi^2_1 = 0.42$, $P = 0.52$). Lastly, we found that outbred and inbred females were equally eager to mate, as measured by the number of total copulations they had over the course of the 45 min choice test (Mann-Whitney U test: $W = 81$, $P = 0.18$).

DISCUSSION

We found that female mating preferences for outbred versus inbred males were conditional upon the female's own inbreeding status: inbred females preferred outbred males over inbred males, whereas outbred females did not show a preference between outbred and inbred males. Inbred females not only copulated with the outbred male more often than with the inbred male (Fig. 1) but most inbred females also mated exclusively with the outbred male (Fig. 2). Our results highlight the potential importance of male inbreeding status as a factor influencing female choice and

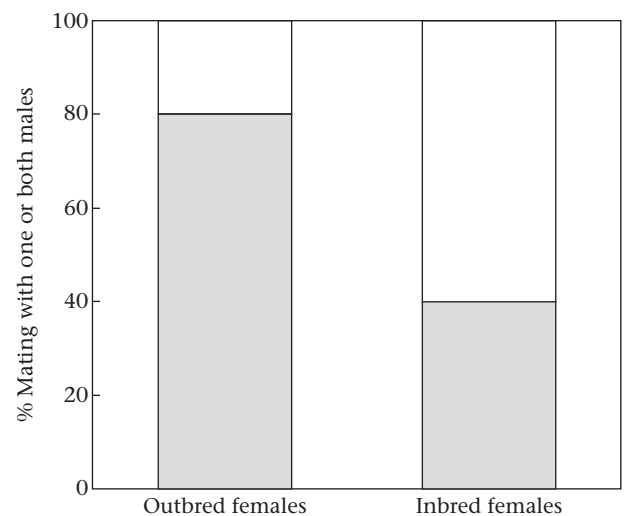


Figure 2. Percentage of outbred and inbred females that mated with only one of the two males (white) or both males (grey) over the course of the 45 min mate choice trials.

demonstrate that female choice may depend on the female's own inbreeding status. Below, we discuss possible explanations for our findings and their wider implications for female mate choice and male mating success in other species.

We found that inbred females showed a mating preference for outbred males over inbred males, whereas outbred females showed no such preference. Given that choosiness is thought to be costly (Pomiankowski, 1987), our results suggest that inbred females may be prepared to pay the costs of being choosy to gain higher marginal benefits (Bolund et al., 2010; Mazzi, Kunzler, Largiader, & Bakker, 2004). Theoretical models predict only small indirect (genetic) benefits to mating with outbred over inbred males (Lehmann et al., 2007; Reinhold, 2002), because mating with an unrelated partner restores offspring heterozygosity regardless of whether that partner is inbred or outbred. Thus, the observed preference of inbred females for outbred males is more likely to be due to direct benefits (Fox et al., 2012).

One direct benefit that plays a role in mate choice in many species is parental care (Johnstone, Reynolds, & Deutsch, 1996; Møller & Jennions, 2001). In burying beetles, males often assist the female in providing care to the offspring, by removing any fur or feathers from the carcass, applying antimicrobials to prevent bacterial and fungal growth, protecting the brood from predators and conspecifics and provisioning the larvae with predigested carrion (Arce, Johnston, Smiseth, & Rozen, 2012; Eggert, Reinking, & Müller, 1998; Pilakouta, Richardson, & Smiseth, 2015; Rozen, Englemoer, & Smiseth, 2008; Walling, Stamper, Salisbury, & Moore, 2008). However, it is unlikely that the observed preference of inbred *N. vespilloides* females for outbred males is driven by a direct benefit of paternal care. This is because the opportunity for females to choose their social partner is restricted by male–male competition over ownership of the carcass. Vertebrate carcasses suitable for breeding are relatively scarce in the wild, so it is common for multiple male and female burying beetles to arrive on a carcass at the same time, resulting in fierce intrasexual competition (Otronen, 1988). Thus, if the female's preferred mate is defeated by another male and driven away from the carcass, he will not provide any care for the resulting offspring.

Instead, it is more likely that the mating preferences we observed were driven by another type of direct benefit, such as sperm number or quality. Inbred males tend to transfer less sperm during copulations and their sperm is less motile and has more abnormalities, leading to lower fertilization success (Ala-Honkola et al., 2013; Fitzpatrick & Evans, 2014; Zajitschek, Lindholm, Evans, & Brooks, 2009). Inbred and outbred females may also produce eggs of different quality, which may be differentially affected by low- versus high-quality sperm produced by inbred and outbred males, respectively. We suggest that the lower sperm quality of inbred males might be more detrimental to fertilization success if the female is also inbred, but to our knowledge, this has not yet been tested. We encourage future research to investigate whether there is an interaction between male and female inbreeding status on fertilization success.

Although the avoidance of inbred males by inbred females might have evolved in direct response to inbreeding, another possibility is that it reflects a general response to an overall decline in condition due to inbreeding depression. Inbreeding is expected to be rare in this species, so it seems unlikely that the mating preferences we observed evolved in the specific context of inbreeding (Mattey & Smiseth, 2015b; Pilakouta, Jamieson et al., 2015). Instead, these mating preferences may be mediated through pre-existing mechanisms that evolved to serve an adaptive function in a different context. For example, females might have evolved general mating preferences for high-quality males, which may be conditional upon their own quality. All populations are

potentially at risk of inbreeding in the future, given increasing habitat loss and other human-induced disturbances that increase the chances of inbreeding (Andersen, Fog, & Damgaard, 2004). Whenever species with no prior history of inbreeding depression become subject to inbreeding, the associated fitness costs may be mediated through pre-existing mechanisms that evolved outside this context (Mattey & Smiseth, 2015b; Pilakouta, Jamieson et al., 2015).

The fact that inbred females preferentially mated with outbred males suggests that females responded to a cue that differentiated inbred and outbred males, such as cuticular hydrocarbons (CHCs) or other chemical cues (Howard & Blomquist, 2005). In insects, CHCs are often used to discriminate between relatives and nonrelatives (Howard & Blomquist, 2005; Tsutsui, 2004; Weddle, Hunt, & Sakaluk, 2013). More specifically, in burying beetles, CHCs are used for partner recognition based on information about sex and breeding status (Müller, Eggert, & Elsner, 2003; Steiger, Peschke, & Müller, 2007), as well as for parent–offspring discrimination (Smiseth, Andrews, Brown, & Prentice, 2010). Females might have been under selection to differentiate between males based on their CHC profiles specifically as a mechanism to avoid mating with inbred males, or as a more general mechanism to avoid mating with males that are in poor condition. Our suggestion that female burying beetles use CHCs to discriminate between outbred and inbred males is in line with a recent study in the butterfly *Bicyclus anynana* showing that inbreeding reduces the production of a male sex pheromone, thereby allowing females to discriminate between males based on their inbreeding status (van Bergen, Brakefield, Heuskin, Zwaan, & Nieberding, 2013). Similarly, there is evidence that female discrimination between outbred and inbred males in mealworm beetles is odour-based (Pölkki et al., 2012). Given that there is a genetic basis to CHCs (Dronnet, Lohou, Christides, Bagnères, 2006; Ferveur, 2005; Foley, Chenoweth, Nuzhdin, & Blows, 2007) and that traits with a genetic basis are prone to inbreeding (van Bergen et al., 2013), CHCs are a plausible mechanism for discrimination between outbred and inbred individuals in *N. vespilloides* and many other insects.

We believe that our findings could have important implications for male mating success in the wild. Earlier work has shown that inbred males often suffer reduced mating success (Ala-Honkola, Uddstrom, Diaz Pauli, & Lindstrom, 2009; Enders & Nunney, 2010; Joron & Brakefield, 2003; Ketola & Kotiaho, 2010; Mariette, Kelley, Brooks, & Evans, 2006; van Oosterhout et al., 2003). Here, we demonstrate that inbred females avoid mating with inbred males but outbred females do not. This suggests that inbred males suffer reduced mating success only when interacting with inbred females. We therefore propose that in species in which female inbreeding status influences mate choice for outbred versus inbred males, the fitness costs of inbreeding with respect to male mating success may be frequency dependent. In populations with high rates of inbreeding, a larger proportion of breeding females will be inbred, and we would expect inbred males to experience lower mating success than in populations with low rates of inbreeding. Such social effects on inbreeding depression in male mating success may be widespread, but their occurrence is still largely unexplored. We encourage future research to further investigate this issue, as it could have important implications for the rate and direction of sexual selection in populations that are subject to inbreeding. For example, under a scenario where inbred females are choosier than outbred females, directional selection on male sexual traits will be stronger when inbreeding rates are high than when they are low.

In summary, we have shown that a female's mating bias for an outbred versus an inbred male depends on her own inbreeding status. This is the first example of a species in which inbred females discriminate against inbred males while outbred females show no

preference between inbred and outbred males. Our findings suggest that inbred females may gain more direct benefits from mating with an outbred male than outbred females do. Lastly, in species in which female inbreeding status influences mate choice for outbred versus inbred males, the fitness costs of inbreeding with respect to male mating success may depend on the frequency of inbred females relative to outbred females and thus the rate of inbreeding in the population.

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References

- Ala-Honkola, O., Hosken, D. J., Manier, M. K., Lupold, S., Droge-Young, E. M., Berben, K. S., et al. (2013). Inbreeding reveals mode of past selection on male reproductive characters in *Drosophila melanogaster*. *Ecology and Evolution*, 3, 2089–2102.
- Ala-Honkola, O., Laine, L., Pekkala, N., Kotiaho, J. S., Honkola, T., & Puurtinen, M. (2015). Males benefit from mating with outbred females in *Drosophila littoralis*: Male choice for female genetic quality? *Ethology*, 121, 577–585.
- Ala-Honkola, O., Uddstrom, A., Diaz Pauli, B., & Lindstrom, K. (2009). Strong inbreeding depression in male mating behaviour in a poeciliid fish. *Journal of Evolutionary Biology*, 22, 1396–1406.
- Andersen, L. W., Fog, K., & Damgaard, C. (2004). Habitat fragmentation causes bottlenecks and inbreeding in the European tree frog (*Hyla arborea*). *Proceedings of the Royal Society B: Biological Sciences*, 271, 1293–1302.
- Andersson, M. (1994). *Sexual selection*. Princeton, New Jersey: Princeton University Press.
- Arce, A. N., Johnston, P. R., Smiseth, P. T., & Rozen, D. E. (2012). Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *Journal of Evolutionary Biology*, 25, 930–937.
- Bartlett, J., & Ashworth, C. M. (1988). Brood size and fitness in *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Behavioral Ecology and Sociobiology*, 22, 429–434.
- van Bergen, E., Brakefield, P. M., Heuskin, S., Zwaan, B. J., & Nieberding, C. M. (2013). The scent of inbreeding: A male sex pheromone betrays inbred males. *Proceedings of the Royal Society B: Biological Sciences*, 280, 20130102.
- Bolund, E., Martin, K., Kempenaers, B., & Forstmeier, W. (2010). Inbreeding depression of sexually selected traits and attractiveness in the zebra finch. *Animal Behaviour*, 79, 947–955.
- Burley, N. T., & Foster, V. S. (2006). Variation in female choice of mates: Condition influences selectivity. *Animal Behaviour*, 72, 713–719.
- Charlesworth, D., & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics*, 18, 237–268.
- Cotton, S., Small, J., & Pomiankowski, A. (2006). Sexual selection and condition-dependent mate preferences. *Current Biology*, 16, 755–765.
- Drayton, J. M., Milner, R. N. C., Hunt, J., & Jennions, M. D. (2010). Inbreeding and advertisement calling in the cricket *Teleogryllus commodus*: Laboratory and field experiments. *Evolution*, 64, 3069–3083.
- Dronnet, S., Lohou, C., Christides, J.-P., & Bagnères, A.-G. (2006). Cuticular hydrocarbon composition reflects genetic relationship among colonies of the introduced termite *Reticulitermes santonensis* Feytaud. *Journal of Chemical Ecology*, 32, 1027.
- Eggert, A.-K., Reinking, M., & Müller, J. K. (1998). Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour*, 55, 97–107.
- Enders, L. S., & Nunney, L. (2010). Sex-specific effects of inbreeding in wild-caught *Drosophila melanogaster* under benign and stressful conditions. *Journal of Evolutionary Biology*, 23, 2309–2323.
- Ferveur, J.-F. (2005). Cuticular hydrocarbons: Their evolution and roles in *Drosophila* pheromonal communication. *Behavior Genetics*, 35, 279.
- Fitzpatrick, J. L., & Evans, J. P. (2014). Postcopulatory inbreeding avoidance in guppies. *Journal of Evolutionary Biology*, 27, 2585–2594.
- Foley, B., Chenoweth, S. F., Nuzhdin, S. V., & Blows, M. W. (2007). Natural genetic variation in cuticular hydrocarbon expression in male and female *Drosophila melanogaster*. *Genetics*, 175, 1465–1477.
- Fox, C. W., Xu, J., Wallin, W. G., & Curtis, C. L. (2012). Male inbreeding status affects female fitness in a seed-feeding beetle. *Journal of Evolutionary Biology*, 25, 29–37.
- Frommen, J. G., & Bakker, T. C. M. (2006). Inbreeding avoidance through non-random mating in sticklebacks. *Biology Letters*, 22, 232–235.
- Gerlach, G., & Lysiak, N. (2006). Kin recognition and inbreeding avoidance in zebrafish, *Danio rerio*, is based on phenotype matching. *Animal Behaviour*, 71, 1371–1377.
- Hansson, B., Jack, L., Christians, J. K., Pemberton, J., Akesson, M., Westerdahl, H., et al. (2007). No evidence for inbreeding avoidance in a great reed warbler-population. *Behavioral Ecology*, 18, 157–164.
- House, C. M., Evans, G. M. V., Smiseth, P. T., Stamper, C. E., Walling, C. A., & Moore, A. J. (2008). The evolution of repeated mating in the burying beetle, *Nicrophorus vespilloides*. *Evolution*, 62, 2004–2014.
- Howard, R. W., & Blomquist, G. J. (2005). Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annual Review of Entomology*, 50, 371–393.
- Hunt, J., Brooks, R., & Jennions, M. D. (2005). Female mate choice as a condition-dependent life-history trait. *American Naturalist*, 166, 79–92.
- Ilmonen, P., Stundner, G., Thoss, M., & Penn, D. J. (2009). Females prefer the scent of outbred males: Good-genes-as-heterozygosity? *BMC Evolutionary Biology*, 9, 104.
- Johnstone, R. A., Reynolds, J. D., & Deutsch, J. C. (1996). Mutual mate choice and sex differences in choosiness. *Evolution*, 50, 1382–1391.
- Joron, M., & Brakefield, P. M. (2003). Captivity masks inbreeding effects on male mating success in butterflies. *Nature*, 424, 191–194.
- Ketola, T., & Kotiaho, J. S. (2010). Inbreeding, energy use and sexual signaling. *Evolutionary Ecology*, 24, 761–772.
- Kokko, H., & Ots, I. (2006). When not to avoid inbreeding. *Evolution*, 60, 467–475.
- Lehmann, L., Keller, L. F., & Kokko, H. (2007). Mate choice evolution, dominance effects, and the maintenance of genetic variation. *Journal of Theoretical Biology*, 244, 282–295.
- Mariette, M., Kelley, J. L., Brooks, R., & Evans, J. P. (2006). The effects of inbreeding on male courtship behaviour and coloration in guppies. *Ethology*, 112, 807–814.
- Mattey, S. N., & Smiseth, P. T. (2015b). Complex effects of inbreeding on biparental cooperation. *American Naturalist*, 185, 1–12.
- Mattey, S. N., & Smiseth, P. T. (2015a). No inbreeding avoidance by female burying beetles regardless of whether they encounter males simultaneously or sequentially. *Ethology*, 121, 1031–1038.
- Mattey, S. N., Strutt, L., & Smiseth, P. T. (2013). Intergenerational effects of inbreeding in *Nicrophorus vespilloides*: Offspring suffer fitness costs when either they or their parents are inbred. *Journal of Evolutionary Biology*, 26, 843–853.
- Mazzi, D., Kunzler, R., Lurgiader, C. R., & Bakker, T. C. M. (2004). Inbreeding affects female preference for symmetry in computer-animated sticklebacks. *Behavior Genetics*, 34, 417–424.
- McKee, A. A., Newton, S. M., & Carter, A. J. R. (2014). Influence of inbreeding on female mate choice in two species of *Drosophila*. *Journal of Insect Behavior*, 27, 613–625.
- Michalczyk, L., Martin, O. Y., Millard, A. L., Emerson, B. C., & Gage, M. J. G. (2010). Inbreeding depresses sperm competitiveness, but not fertilization or mating success in male *Tribolium castaneum*. *Proceedings of the Royal Society B: Biological Sciences*, 277, 3483–3491.
- Møller, A. P., & Jennions, M. D. (2001). How important are direct fitness benefits of sexual selection? *Naturwissenschaften*, 88, 401–415.
- Müller, J. K., Eggert, A.-K., & Elsner, T. (2003). Nestmate recognition in burying beetles: The 'breeder's badge' as a cue used by females to distinguish their mates from male intruders. *Behavioral Ecology*, 14, 212–220.
- Neff, B. D., & Pitcher, T. E. (2008). Mate choice for nonadditive genetic benefits and the maintenance of genetic diversity in song sparrows. *Journal of Evolutionary Biology*, 22, 424–429.
- Nietlisbach, P., Keller, L. F., & Postma, E. (2016). Genetic variance components and heritability of multiallelic heterozygosity under inbreeding. *Heredity*, 116, 1–11.
- Okada, K., Blount, J. D., Sharma, M. D., Snook, R. R., & Hosken, D. J. (2011). Male attractiveness, fertility and susceptibility to oxidative stress are influenced by inbreeding in *Drosophila simulans*. *Journal of Evolutionary Biology*, 24, 363–371.
- van Oosterhout, C., Trigg, R. E., Carvalho, G. R., Magurran, A. E., Hauser, L., & Shaw, P. W. (2003). Inbreeding depression and genetic load of sexually selected traits: How the guppy lost its spots. *Journal of Evolutionary Biology*, 16, 273–281.
- Otronen, M. (1988). The effect of body size on the outcome of fights in burying beetles (*Nicrophorus*). *Annales Zoologici Fennici*, 25, 191–201.
- Pilakouta, N., Jamieson, S., Moorad, J. A., & Smiseth, P. T. (2015). Parental care buffers against inbreeding depression in burying beetles. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 8031–8035.
- Pilakouta, N., Richardson, J., & Smiseth, P. T. (2015). State-dependent cooperation in burying beetles: Parents adjust their contribution towards care based on both their own and their partner's size. *Journal of Evolutionary Biology*, 28, 1965–1974.
- Pilakouta, N., Sieber, D., & Smiseth, P. T. (2016). Sibling competition does not exacerbate inbreeding depression in the burying beetle *Nicrophorus vespilloides*. *Journal of Evolutionary Biology*, 29, 704–710.
- Pilakouta, N., & Smiseth, P. T. (2016). Maternal effects alter the severity of inbreeding depression in the offspring. *Proceedings of the Royal Society B: Biological Sciences*, 283, 20161023.
- Pölkki, M., Krams, I., Kangassalo, K., & Rantala, M. J. (2012). Inbreeding affects sexual signalling in males but not females of *Tenebrio molitor*. *Biology Letters*, 8, 423–425.
- Pomiankowski, A. (1987). The costs of choice in sexual selection. *Journal of Theoretical Biology*, 128, 195–218.
- Pusey, A. E., & Wolf, M. (1996). Inbreeding avoidance in animals. *Trends in Ecology and Evolution*, 11, 201–206.

- R Core Team. (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <http://www.R-project.org/>.
- Reinhold, K. (2002). Modelling the evolution of female choice strategies under inbreeding conditions. *Genetica*, *116*, 189–195.
- Rozen, D. E., Englemoer, D. J. P., & Smiseth, P. T. (2008). Antimicrobial strategies in burying beetles breeding on carrion. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 17890–17895.
- Smiseth, P. T., Andrews, C., Brown, E., & Prentice, P. M. (2010). Chemical stimuli from parents trigger larval begging in burying beetles. *Behavioral Ecology*, *21*, 526–531.
- Smiseth, P. T., Dawson, C., Varley, E., & Moore, A. J. (2005). How do caring parents respond to mate loss? Differential response by males and females. *Animal Behaviour*, *69*, 551–559.
- Smiseth, P. T., & Moore, A. J. (2004). Behavioral dynamics between caring males and females in a beetle with facultative biparental care. *Behavioral Ecology*, *15*, 621–628.
- Steiger, S., Peschke, K., & Muller, J. K. (2007). Correlated changes in breeding status and polyunsaturated cuticular hydrocarbons: The chemical basis of nestmate recognition in the burying beetle *Nicrophorus vespilloides*? *Behavioral Ecology and Sociobiology*, *62*, 1053–1060.
- Szulkin, M., Stopher, K. V., Pemberton, J. M., & Reid, J. M. (2013). Inbreeding avoidance, tolerance, or preference in animals? *Trends in Ecology and Evolution*, *28*, 205–211.
- Tregenza, T., & Wedell, N. (2000). Genetic compatibility, mate choice and patterns of parentage: Invited review. *Molecular Ecology*, *9*, 1013–1027.
- Trumbo, S. T. (1996). The role of conflict in breeding systems: Burying beetles as experimental organisms. *American Biology Teacher*, *58*, 118–121.
- Tsutsui, N. D. (2004). Scents of self: The expression component of self/non-self recognition systems. *Annales Zoologici Fennici*, *41*, 713–727.
- Walling, C. A., Stamper, C. E., Salisbury, C. L., & Moore, A. J. (2008). Experience does not alter alternative mating tactics in the burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology*, *20*, 153–159.
- Weddle, C. B., Hunt, J., & Sakaluk, S. K. (2013). Self-referent phenotype matching and its role in female mate choice in arthropods. *Current Zoology*, *59*, 239–248.
- Zajitschek, S. R. K., & Brooks, R. C. (2010). Inbreeding depression in male traits and preference for outbred males in *Poecilia reticulata*. *Behavioral Ecology*, *21*, 884–891.
- Zajitschek, S. R. K., Lindholm, A. K., Evans, J. P., & Brooks, R. C. (2009). Experimental evidence that high levels of inbreeding depress sperm competitiveness. *Journal of Evolutionary Biology*, *22*, 1338–1345.