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Exposure time to rivals and sensory cues affect how quickly males respond to changes in sperm competition threat

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Phenotypic plasticity can increase fitness in rapidly changeable environments, but may be limited if the underlying mechanisms cause a lag between environmental change and individual response or if the information individuals receive is unreliable. Hence to understand the evolution of plasticity we need to assess whether individuals respond to fine-scale variation in environmental cues. In this study we used a *Drosophila melanogaster* fruit fly model to investigate factors that determine how quickly males alter their behaviour in response to changes in sperm competition cues. Male *D. melanogaster* respond to exposure to rival males prior to mating by extending mating duration and increasing ejaculate investment. We have previously shown that to build-up the response, males need about 24 h exposure to a rival. We reasoned that this time lag was necessary to increase ejaculate production, but this physiological limitation should not

apply when moving from high- to low-competition environments; hence we predicted that males should immediately decrease their investment when competition is removed. Here we tested this by measuring how long rival-exposed males maintained an extended mating duration after removal of the rival. We assessed how exposure time and sensory information affected the speed of change in behavioural state. Males maintained extended mating duration for hours after a rival was removed, but this was dependent on time of exposure to a rival. Furthermore, although sensory-impaired males were able to respond to rivals, the time required for the response to build and diminish depended on males possessing their full sensory repertoire. Our results suggest that males use exposure time and multiple sensory cues to assess whether the threat of sperm competition is transient (so unlikely to translate into realized competition) or sustained (requiring a response). Therefore, time lags between environmental changes and responses may buffer animals against making hasty decisions in fluctuating environments.

Key Words: behavioural plasticity, *Drosophila melanogaster,* information reliability, learning, memory, time lag limit

Phenotypic plasticity is the expression of different phenotypes from the same genotype in response to an environmental cue (West-Eberhard 2003). In animals, behavioural plasticity is predicted to be a particularly potent form of phenotypic plasticity due to its rapid flexibility and low production costs (Parker 1982), and hence flexible behaviour can enable animals to cope with fluctuating environments (Komers 1997). However, to be adaptive, behavioural plasticity must track the environment accurately and on a similar timescale to the environmental variation to which it responds (Gabriel et al. 2005). If it does not, mismatches between behaviour and the environment are predicted to be costly (Auld, Agrawal & Relyea 2010). For example, there is growing evidence that climate change is currently driving phenological mismatches in reproduction (Reale et al. 2003), development of seasonal camouflage (Mills et al. 2013), hibernation emergence (Ozgul et al. 2010; Lane et al. 2012) and migration (Both & Visser 2001). Clearly, gaining accurate information in order to predict future environments is essential, and this requires sensory systems that can assimilate environmental information. Moreover, depending on the type of environmental variation, the proximate cues might change more quickly than the prevailing population conditions, and so animals might need to judge whether the change is transient or sustained enough to warrant a response. We therefore need to assess whether individuals respond to fine-scale variation in environmental cues.

One rapidly changing facet of the environment is the sociosexual context, as sex ratio can vary locally and over short timescales (Kasumovic et al. 2008; Punzalan, Rodd & Rowe 2010). This is particularly important for males as they are predicted to allocate reproductive resources strategically, trading off current and future mating opportunities depending on the competitive environment (Parker et al. 1996; Parker et al. 1997). Plastic mating strategies in response to changing sociosexual environments are well documented, with males strategically allocating ejaculates (Wedell, Gage & Parker 2002) and/or adjusting behaviour (Bretman, Gage & Chapman 2011). Some of these strategies are an immediate response to another male (or cues of other males) present at the time of mating; others require a period of exposure to a rival beforehand, although few studies are designed to measure both (Bretman, Gage & Chapman 2011). We currently have very little understanding of how males assess and assimilate environmental information and how this is translated into altered behavioural and physiological states. One of the best studied examples is the response of male *Drosophila melanogaster* fruit flies, whereby males exposed to a rival male before mating subsequently mate for longer than males held alone (Bretman, Fricke & Chapman 2009). This leads to increased short-term

reproductive success compared to males that have not been exposed to rivals (Bretman, Fricke & Chapman 2009), mediated by alterations in ejaculate contents (Wigby et al. 2009; Garbaczewska, Billeter & Levine 2013; Moatt, Dytham and Thom 2014). Individual males can alter mating duration in either direction, increasing it after exposure to a rival and reducing it when that rival is removed (Bretman et al. 2012). Males kept with rivals die sooner and become progressively less successful at obtaining matings over their lifetimes, supporting the idea that there are costs of responding to rivals (Bretman et al. 2013). Males detect rivals via any paired combination of olfactory, auditory and tactile sensory cues, which implies a system of sensory redundancy and reinforces the idea that making the wrong decision about the appropriate level of investment is costly (Bretman et al. 2011).

In this study, we explored how quickly males respond to a new competitive environment and what factors affect the speed of adjustment. We have previously shown that males require about 24 h exposure to a rival to increase mating duration and gain fitness benefits, and we reasoned this time lag may be required to increase production of ejaculate components (Bretman et al. 2010). However, males moving from a high- to a low-competition environment should not be constrained by this physiological limitation and so should not require any adjustment time. If this is the only consideration in the speed of response, then we predict that males moved from high to low competition should quickly change their strategy and not mate for longer than males that have never perceived competition. We measured how long rival-exposed males continued to extend mating duration after the rival had been removed and how this was affected by the length of exposure time. We also tested how sensory information affected the speed of response to changes in the sperm competition environment by manipulating auditory and olfactory inputs.

<H1>Methods

Experiments were conducted in a 25 °C humidified room with a 12:12 h light:dark cycle, using plastic vials (75x25 mm) with 7 ml standard sugar–yeast–agar medium (Bass et al. 2007). All wild-type flies were the Dahomey strain as in our previous studies. Larvae were raised at a standard density of 100 per vial. At eclosion, flies were collected and sexed using ice anaesthesia, and stored 10 per vial. Females were supplemented with live yeast granules. Males were aged for 24 h before being randomly assigned to a social

environment treatment, i.e. plus-rival or no-rival, with a starting $N = 40$ for all groups. In different experiments we manipulated 'exposure time' (time from introduction to removal of the rival) and 'maintenance time' (time from removal of the rival to mating; Fig 1, Table 1). At mating, males were aspirated singly into a vial containing a single female and allowed to mate, and mating duration was recorded. If no mating occurred within 2 h the vial was discarded; hence these plus any losses during transfers reduced the final N for each group (see figures for sample sizes).

<H2>Effect of exposure time on maintenance time of extended mating duration

In experiment 1, we investigated how the response to a rival in terms of extended mating duration was maintained over the 48 h after the rival was removed (maintenance time). This required offsetting the introduction of the rival and therefore the day on which males were mated. Hence, we set up paired treatments, whereby each plus-rival treatment had a corresponding no-rival treatment handled in the same way. The plus-rival treatments were exposed to a rival for 72 h to make sure the full response was achieved (Bretman et al. 2010), and then isolated for 0, 12, 24, 36 or 48 h before mating. In experiment 2 we further narrowed down the maintenance time. Here we were able to mate all males at once; hence we had one no-rival treatment and seven plus-rival treatments exposed to a rival for 72 h and then isolated for 0, 9, 12, 15, 18, 21 or 24 h before mating.

To test whether the amount of time males spent with rivals (exposure time) affected the maintenance time of the response we repeated experiment 1, but this time plus-rival treatments were exposed to a rival for either 36 h (experiment 3) or 24 h (experiment 4). After exposure, focal males were isolated for 0, 12 or 24 h prior to mating. Finding that 24 h exposure reduced maintenance time we further narrowed this down as in experiment 2, this time giving plus-rival treatments 24 h exposure and isolating them for 0, 1, 3, 6, 9 or 12 h before mating and comparing them to a single no-rival treatment (experiment 5).

<H2>Effect of sensory deprivation on speed of behavioural response

To investigate whether sensory deprivation would affect maintenance or build-up of extended mating duration, we manipulated olfactory and auditory cues as in our previous work (Bretman et al. 2011). To

remove auditory signals rival male wings were removed under CO_2 anaesthesia. We removed olfaction by, using as focal males *Orco*^{2} (formally *odorant receptor 83b*) mutants lacking a co-receptor responsible for perceiving 80% of *D. melanogaster*'s odour range (Larsson et al. 2004). We also used wild-type males with their third segment of antennae removed under CO_2 anaesthesia, which removes sensillae required for males to respond to odour cues (van Naters & Carlson 2007), and also aristae which contribute to detection of sound (Gopfert & Robert 2002). All sensory manipulations were performed before males were assigned to their social treatment. We repeated experiment 1 but with focal males in the plus-rival treatment isolated for 0, 12 and 24 h after mating. We also measured the effect of these sensory manipulations on the build-up of the mating duration response over 29 h (experiment 7). Males were collected singly before being exposed to rivals for 20, 24 and 29 h prior to mating. Importantly, in all experiments, comparisons were only made between males with the same sensory manipulation kept singly or with a rival, hence controlling for manipulation or genetic background effects.

<H2>Statistical analysis

Statistical analysis was performed using SPSSv14 (IBM, Armonk, NY, U.S.A.). If data were normally distributed, comparisons between three or more treatments were made using ANOVA with Dunnett's post hoc tests and pairs of treatments using *t* tests. If data did not meet the assumptions of these tests, then Kruskal - Wallis or Mann–Whitney *U* tests were used (as indicated in the Results section). To reiterate, where the design permitted, the key comparisons were between males kept singly or with a rival but treated the same in all other respects, as this controlled for any other manipulation effects. Bonferroni corrections were made where multiple tests were used.

<H2>Ethical Note

Our study involved *D. melanogaster* that had been maintained exclusively under laboratory conditions for several hundred generations. As they are invertebrates, they are not subject to any special ethical requirements; however every effort was made to minimize discomfort. All physical manipulations were

performed under light CO_2 or ice anaesthesia, and the fly was given 24 h to recover until any further social manipulations were performed.

<H1>Results

<H2>Effect of exposure time on maintenance time of extended mating duration

In experiment 1, after 72 h exposure to a rival, males extended mating duration for 12 h (Mann–Whitney *U* test: $Z = -3.722$, $N = 77$, $P < 0.001$), but not for 24 h ($t_{70} = -1.597$, $P = 0.115$) or more after removal of the rival (Fig. A1a). In experiment 2, we narrowed maintenance time down further, again finding that mating duration was affected by time since isolation from a rival (Kruskal–Wallis: $\chi^2_7 = 15.862$, $P = 0.026$). Post hoc tests showed that males continued to significantly increase mating duration after 12 h of isolation (Mann–Whitney *U* test: $Z = -3.136$, $N = 77$, $P = 0.014$), but failed to do so after 15 h isolation (Mann–Whitney *U* test: $Z = -2.349$, $N = 75$, $P = 0.133$; Fig. A1b).

Length of exposure to a rival affected the maintenance of extended mating duration. Males exposed to rivals for 36 h showed a similar pattern to those exposed for 72 h (experiment 1) and extended mating duration for at least 12 h after removal of the rival (experiment 3; Mann–Whitney *U* test: $Z = -3.294$, $N = 76$, $P = 0.001$; Fig. 2a). This was not the case for males that had only been exposed to a rival for 24 h before isolation (experiment 4; Mann–Whitney *U* test: $Z = -0.985$, $N = 71$, $P = 0.324$; Fig. 2b). We explored this further, finding that when males had been exposed to a rival for 24 h (experiment 5) only males isolated for 0 h (Mann–Whitney *U* test: $Z = -3.292$, $N = 75$, $P = 0.006$) and 1 h (Mann–Whitney *U* test: $Z = -3.406$, $N = 72$, $P = 0.006$) before mating mated for significantly longer than males never exposed to a rival (Fig. 2c).

<H2>Effect of sensory deprivation on speed of behavioural response

In experiment 6, we tested how sensory manipulations modulated the maintenance time of extended mating duration. Males exposed to rivals but not receiving auditory (wing-removed rivals) or olfactory cues (use of *Orco*^{2} mutants) showed a pattern similar to that in unmanipulated wild-type flies. These males increased their mating duration for 12 h after removal of the rival (wing removal: Mann–Whitney *U* test: $Z = -2.812$, $N = 73$,

P= 0.005; Fig. 3a; *Orco*^{2}: Mann–Whitney U test: Z = 2.388, N = 58, P = 0.017; Fig. 3b), but not after 24 h isolation (wing removal: t_{73} = -0.659, P = 0.512; Fig. 3a; *Orco*^{2}: t_{51} = -1.124, P = 0.266; Fig. 3b). In contrast, when the third antennal segment was removed, males continued to extended mating duration for 24 h (Mann–Whitney U test: Z = -2.891, N = 66, P = 0.004; Fig. 3c), ca. 10 h longer than unmanipulated wild-type males.

We also investigated how sensory manipulations affected the speed with which males built up a response to rivals. Our previous work showed that males can respond after 24 h exposure time, confirmed here in experiments 3 and 4. However, in each of our sensory manipulations we found no significant increase in mating duration even after 29 h exposure to a rival (wing removal: Kruskal–Wallis: χ^2_3 = 7.774, P = 0.500; Fig. 4a; *Orco*^{2}: ANOVA: $F_{3, 80}$ = 1.302, P = 0.280; Fig. 4b; third segment: ANOVA: $F_{3, 94}$ = 1.589, P = 0.197; Fig. 4c). This suggests that although males can respond when sensory cues are removed, sensory deprivation increases the time lag between the environmental change and the behavioural response.

<H1>Discussion

We have shown that the speed with which males can adjust their behaviour to a new sperm competitive environment is dictated both by the length of time exposed to a rival and the type of sensory inputs males receive. Given 36 or 72 h exposure time, the increase in mating duration seen after exposure to a rival male was maintained for 12 h after removal of the rival, in line with a previous report (Kim, Jan & Jan 2012). However, after 24 h exposure, the significant increase in mating duration only persisted for 1 h after removal of the rival. Exposure time therefore altered how long the behavioural response was maintained and that while it is possible for males to alter behaviour shortly after a rival is removed, they do not if they have had at least 36 h exposure time. Removal of auditory or olfactory cues prevented males from responding as quickly to the introduction of a rival compared to males with full sensory abilities, as no significant response was measured after 29 h exposure for any of the sensory manipulations we tested. Single cue manipulations did not alter the pattern of reduction of the response once the rival was removed. However, a manipulation that probably affected both olfaction and hearing (removal of the third antennal segment) caused males to maintain a response for longer after isolation.

Previously, we found it is necessary for males to be exposed to rivals for at least 24 h before displaying an adaptive response, which could be considered a time lag limit to plasticity. We suggested this

time was required to enable a corresponding alteration of ejaculate components (Bretman et al. 2010), such as more seminal proteins (Wigby et al. 2009) or more/better quality sperm (Garbaczewska et al. 2013; Moatt et al. 2014). In *D. melanogaster*, the relationship between mating duration per se and ejaculate transfer is not straightforward (Gilchrist & Partridge 2000; Manier et al. 2010), but multiple studies have shown that its modulation in response to social contact does affect fitness (Bretman et al. 2009, 2011, 2012, 2013; Price et al. 2012). Nevertheless, over successive matings, the duration response and fitness outcomes can become uncoupled (Bretman et al. 2012, 2013), suggesting the behaviour alone does not alter fitness. This is the basis of our prediction that to build up the response requires time to produce more/better quality ejaculate components. However, males could reduce investment by shortening mating duration and transferring less ejaculate, so immediately respond to the removal of competition. This seems unlikely to explain why there is a lag between removal of the rival and the decrease in mating duration. Moreover, after 24 h exposure, males did respond to rivals but quickly reduced mating duration after the rival was removed. Males are therefore capable of rapidly adjusting their behaviour, although here we did not measure whether there is a corresponding speedy adjustment to ejaculate transfer.

Theory generally predicts that males should invest more as sperm cometition risk (probability of a female remating) increases. However, with respect to sperm competition intensity (number of competing ejaculates), investment should be maximized with one rival, and decline thereafter as potential fitness returns diminish with each additional competitor (Parker et al. 1996; Parker et al.1997). However there are many variations to these models incorporating factors such as the quality of information available to the male, female quality and male age, experience and condiditon (Parker & Pizzari 2010). In our *D. melanogaster* example, exposure time might give males information about both risk and intensity. However, our previous work showed that males were not sensitive to the number or density of rivals, i.e. intensity (Bretman et al. 2010), suggesting that the critical determinant of fitness is whether or not a male faces competition (risk), rather than with how many other males. A further consideration is whether males respond to the population mean rather than immediate threat. Longer exposure times might indicate that even though the immediate competitive threat is removed, the competition within the area or population is high and therefore greater investment should be maintained as insurance against sudden increases in competition. Indeed, recent evidence suggests that males can be primed for the average levels of sperm competition within the

population if they receive cues as larvae, which might be 10 days before they become adult and are subject to that competition. Males raised in the presence of adult males or in high larval densities developed larger accessory glands (Bretman et al. 2016) and the latter condition also increased their relative allocation of seminal fluid proteins when adult (Wigby et al. 2015). However, developmental environment was not found to affect adult behavioural strategies, suggesting that cues received as juveniles can accurately predict the average population level of sperm competition but are a relatively poor indicator of immediate competition at any particular mating (Bretman et al. 2016).

Our findings suggest that responding to the addition or removal of rivals immediately may not be the best strategy; hence we might question whether this time lag is a true limit to plasticity or is actually adaptive. If the competitive environment can change rapidly, sensory cues could be misleading, if for example males are not constantly in physical contact but competition may be nearby. For animals such as *D. melanogaster* that are difficult to observe in the wild, it is unlikely we could accurately measure the natural timescale of their social interactions. Nevertheless, it is likely that the environment varies, as without this variation the plastic response should not be maintained or initially evolve (Carroll & Corneli 1995). Given that flies will aggregate around food sources we can speculate that males could spend 72 h in intense social contact (Stamps et al. 2005; Reaume & Sokolowski 2006). Conversely, they could be socially isolated for 12 h or longer, for example when moving between food patches or sheltering from adverse weather conditions, and in this context the ability to remember a previous social contact should be beneficial. We employed manipulations in which males were continuously with or without a rival for given periods and natural fluctuations may be more dynamic. In other contexts, training flies in short bursts leads to greater memory consolidation (Tully et al. 1994), so future work could address whether increased frequency of fluctuations in the social environment increases the maintenance of the extended mating duration response.

Throughout this and much of our previous work, a single fly has been used as a competitor. Although in natural settings it might be expected that multiple males would simultaneously or successively interact, in a laboratory setting neither number nor density of rivals affects the magnitude of the response (Bretman et al. 2010). In addition, all males were virgin; however, we have shown that prior sexual experience does not affect the mating duration response (Bretman et al. 2012). Other studies have shown that males can employ plastic sperm competition strategies depending on female mating status, quality and age.

For example, in *D. melanogaster* males can respond to female mating status by altering sperm number (Lüpold et al. 2011) and seminal fluid composition (Sirot, Wolfner & Wigby 2011), although note that the direction of this response (i.e. more investment in mated or virgin females) is not consistent across studies (Friberg 2006). The mating status of the female has been shown not to affect the extended mating duration in response to rival exposure (Bretman et al. 2009), and females have little ability to control mating length once mating has begun (Bretman et al. 2013). Nevertheless, future work could test whether male experience or age, or female mating status, alters the speed with which males respond to changes in competition cues.

Another hypothesized limit to plasticity is that of information reliability (Auld et al. 2010), specifically that plastic responses require sensory recognition systems that accurately perceive and process environmental information. In our paradigm this means sensing that another individual poses a sperm competition threat, which could require incorporating information about whether the rival is conspecific, male and sexually mature. We have previously suggested that because males lacking one sense are able to respond, this shows sensory redundancy (Bretman et al. 2011). In the current study, while males could still respond to a reduction in competition when we manipulated single senses (either 80% olfaction through use of $Orco^2$ or auditory through removal of rivals' wings), the speed of behavioural response was reduced, suggesting the senses are not fully redundant. It is worth noting though that we did not combine sensory manipulations with our assessment of the effect of exposure time on maintenance time (i.e. did not repeat experiments 1–5 with the three sensory manipulations). In a simple odour associative learning task in *D. melanogaster*, odour detection was not the rate-limiting step in decision making, but when faced with more difficult tasks (distinguishing between low-contrast stimuli), flies took longer to gather information before making a choice (DasGupta, Ferreira & Miesenboeck 2014). Arguably, integrating information from multiple cues to detect the presence or absence of a rival is rather more cognitively challenging than such single–odour tests. Nevertheless, our findings might suggest that in responding to rival males, the rate at which sensory information can be gathered does not impose a limit on plasticity, but the task becomes more difficult (although not impossible) when senses are removed. Moreover, the speed of response patterns are not fully symmetrical; single-sense manipulations affected the build-up of the response but not the decline of the response when the rival was removed. This raises the question of whether the build-up of the response is more sensitive to information reliability limits, perhaps because it is likely to be more costly to make the

wrong decision and build up a response when competition is low than to maintain it once competition has been removed (Bretman et al. 2010).

In contrast to the single-sense manipulations, removal of the third antennal segment affected both build-up and decline of the response. This manipulation probably inhibits both olfaction and hearing (Gopfert & Robert 2002), but probably does not fully remove either sense, as for example *Orco* is also expressed in the maxillary palps (Larsson et al. 2004). It is thought that competitor recognition in general (e.g. direct aggressive conflict over territories) requires multiple cues across different sensory modalities (Grether 2011). In the few examples where cues of sperm competition rivals have been explored, most require only a single auditory (Bailey, Gray & Zuk 2010) or chemical cue (delBarco-Trillo & Ferkin 2004; Carazo, Font & Alfthan 2007; Aragon 2009; Larsdotter-Mellstrom et al. 2016). The only other study so far to report a requirement for multiple cues showed that the fruit fly *Drosophila pseudoobscura* requires both odour and tactile cues (Maguire, Lizé & Price 2015), similar but not identical to *D. melanogaster*. As yet we cannot explain these differences, especially as speed of response has not been considered in these other animals, but this variation shows the evolutionary variability of cue recognition systems (Maguire et al. 2015). Multimodal communication is thought to speed up reaction times (Rowe 1999), but this idea relates to reactions on a timescale of seconds (Zeyl & Laberge 2011) rather than hours as we describe. Whether the multiple cues males use in this context convey different information (e.g. sex or species), or contribute similar information but achieve a response threshold faster, remains to be investigated.

To further our understanding of the neuroecology and evolution of recognition systems, and plasticity in general, we need to examine these processes mechanistically, at neuronal, biochemical and genetic levels. Here *D. melanogaster* offers significant advantages, as it is a well-established model for exploring learning and memory mechanisms. Indeed, in such studies a commonly used assay is that of courtship suppression, whereby male *D. melanogaster* exposed to unreceptive (recently mated) females learn to reduce courtship effort (Kamyshev, Iliadi & Bragina 1999). It is thought that although courtship itself requires various sensory inputs (Krstic, Boll & Noll 2009), suppression is largely learnt through chemical cues (Griffith & Ejima 2009). Similar to the response to rivals, exposure time to female cues is important, but interestingly, discrete training periods rather than constant contact is required for males to consolidate this from short-term to long-term memory (McBride et al. 1999). Although these behaviours show parallels,

they may be cognitively very different tasks: Courtship suppression is somewhat binary (i.e. learning a cue that the female is or is not receptive) whereas responding to rivals requires remembering an amount of time spent with a rival male as a proxy for the probability of future competition. As the pathways controlling courtship suppression are well documented (Griffith & Ejima 2009), it will be fruitful to compare the learning and memory mechanisms involved.

In conclusion, we have shown that in *D. melanogaster*, the speed of behavioural responses to sperm competition rivals is affected by prior exposure time and sensory cues. Behavioural plasticity is thought to be a cheap and fast way to cope with environmental change, yet we have shown that males do not always respond to changes in their competitive environment as quickly as they are able. Our findings could be interpreted as limitations of plasticity, or alternatively that both the time lag and information from the full sensory repertoire allow males to quantify sperm competition threat within a population. These findings could therefore have important implications for understanding context-dependent decision making, especially as this *Drosophila* model will enable future studies to dissect these processes at many mechanistic levels.

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457 Appendix

458

459 Table 1: Description of experiments 1–7 providing information about the exposure and maintenance time for
460 each experiment

Experiment	Paired treatments?*	Exposure time (h)	Maintenance time (h)
Effect of exposure time on maintenance time			
1	y	72	0, 12, 24, 36, 48
2	n	72	0, 9, 12, 15, 18, 21, 24
3	y	36	0, 12, 24
4	y	24	0, 12, 24
5	n	24	0, 1, 3, 6, 9, 12
Effect of sensory deprivation on speed of behavioural response			
6	y	72	0, 12, 24
7	y	20, 24, 29	0

461 * y: each plus-rival treatment has a corresponding no-rival treatment; n: multiple plus-rival treatments
462 compared to one no-rival treatment.

Figure legends

Fig 1: Experimental design. Focal males (solid symbols) were separated at eclosion and haphazardly assigned to no-rival (vials 1) or plus-rival (vial 2, rival is the dotted symbol) treatments, handled in exactly the same way except for the presence of absence of the rival. In different experiments we varied exposure time (time kept with the rival male) and maintenance time (time from removal of the rival male until mating), as described in Table 1. Focal males were transferred to new vials for isolation (vials 1a and 2a) and females were added to these vials to record mating duration.

Fig 2: Effect of exposure time on maintenance time in response to a rival. Mating duration (mean +/- SEM) of males held singly or exposed to a rival for (a) 36 h or (b, c) 24 h. (a and b) Males were held singly (white bars) or exposed to a rival (grey bars) and separated for 0, 12 and 24 h before mating. Final sample sizes for each treatment group are given within the appropriate bar. An asterisk indicates a significant difference between paired treatments: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. (c) Males were held singly or exposed to a rival then separated for 0–24 h before mating. An asterisk indicates a significant difference compared to the single treatment, after Bonferroni correction.

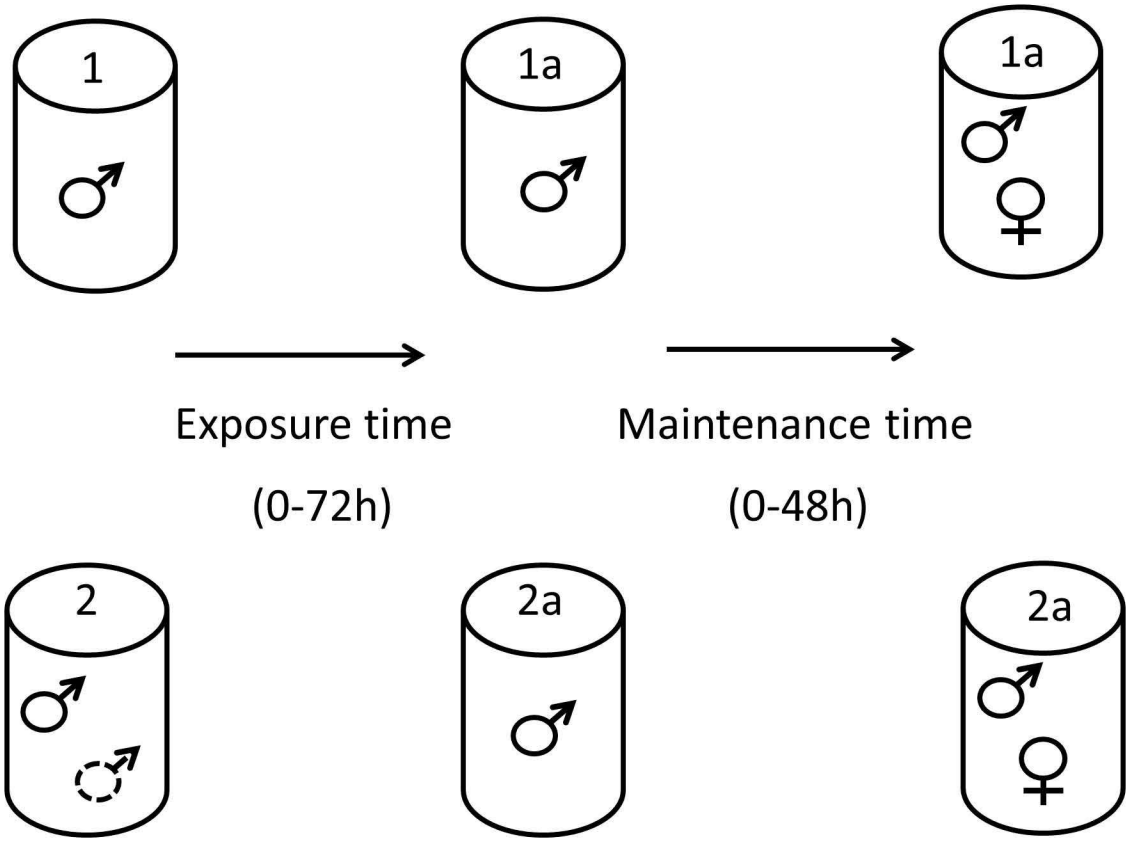
Fig 3: Sensory deprivation effects on maintenance of extended mating duration. Mating duration (mean +/- SEM) of males held singly (white bars) or with rivals (grey bars) for 72 h before being isolated for 0, 12 or 24 h. (a) Males maintained with wingless rivals. (b) *Orco*^{2} focal males lacking odorant co-receptor. (c) Wild-type focal males with the third segment of their antennae removed. Final sample sizes for each treatment group are given within the appropriate bar. An asterisk indicates a significant difference between paired treatments: *** $P < 0.001$.

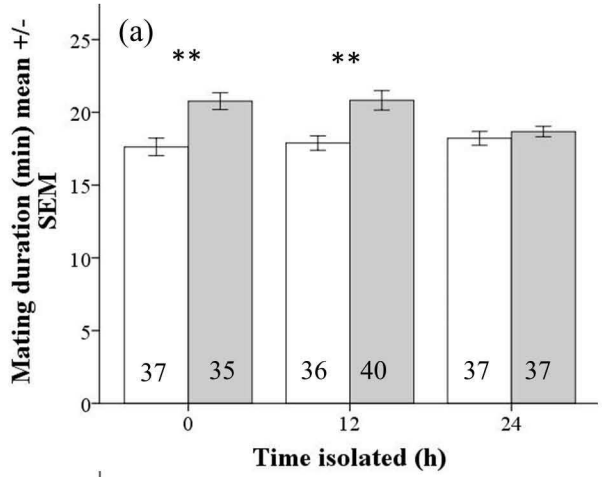
Fig 4: Sensory deprivation effects build-up of sperm competition response. Mating duration (mean +/- SEM) of males held singly (white bars) or with rivals (grey bars) for 20, 24 and 29 h before immediate mating to females. (a) Focal males maintained with wingless rivals. (b) *Orco*^{2} focal males lacking odorant co-receptor.

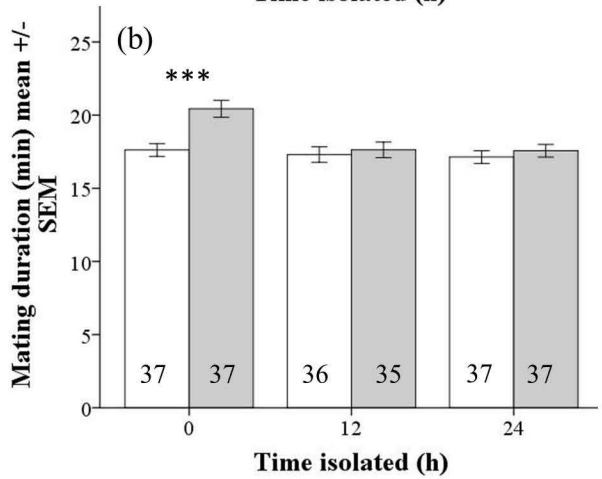
489 (c) Wild-type focal males with the third segment of their antennae removed. Final sample sizes for each
490 treatment group are given within the appropriate bar.

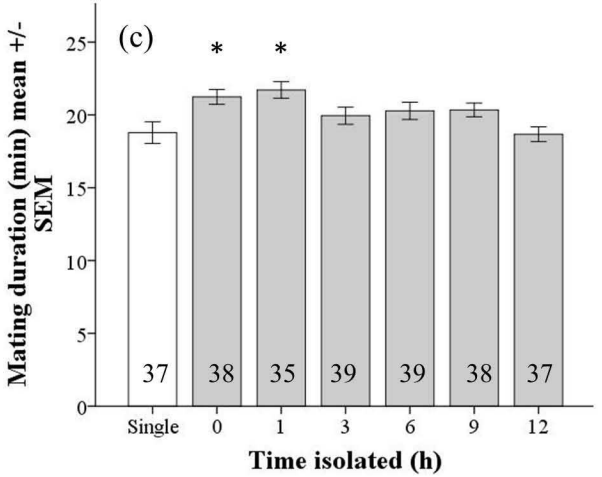
491

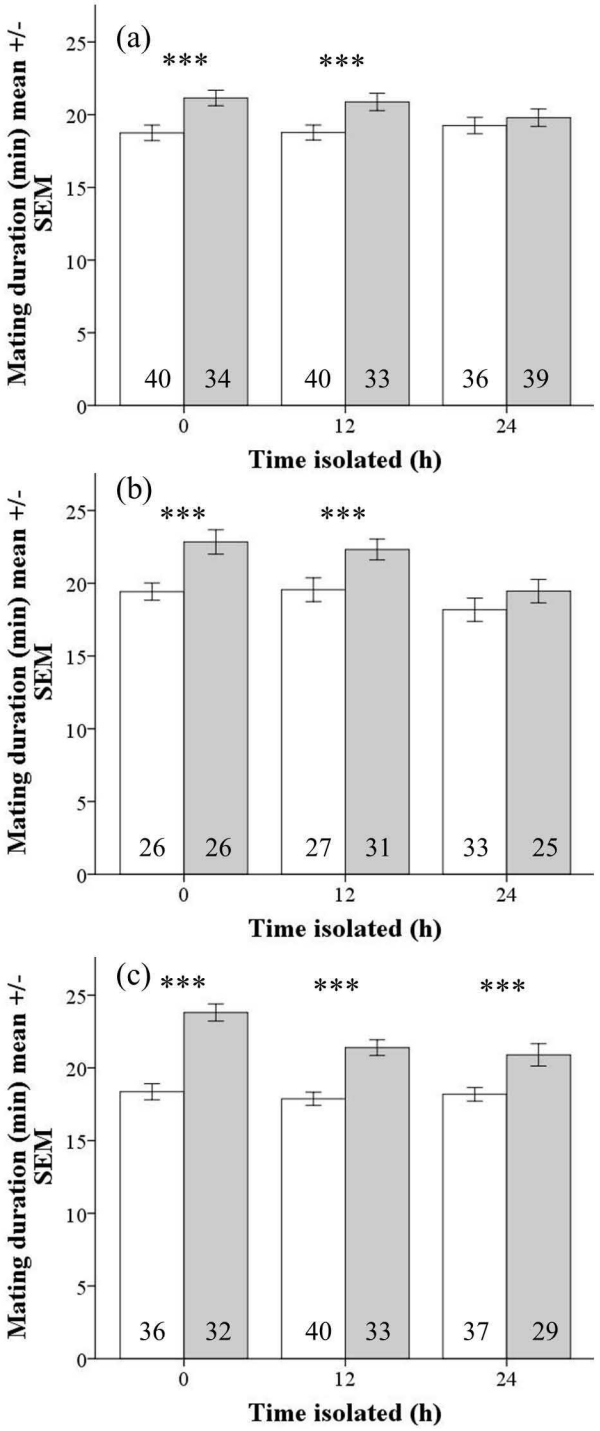
492 Figure A1: Effect of maintenance time on mating duration. Mating duration (mean +/- SEM) of males held
493 singly or exposed to rivals for 72 h. (a) Males were held singly (white bars) or exposed to a rival (grey bars)
494 then separated for 0–48 h before mating. Final sample sizes for each treatment group are given within the
495 appropriate bar. An asterisk indicates a significant difference between paired treatments: ** $P < 0.01$; *** P
496 < 0.001. (b) Males were held singly or exposed to a rival and separated for 0–24 h before mating. An asterisk
497 indicates a significant difference compared to the single treatment, after Bonferroni correction.

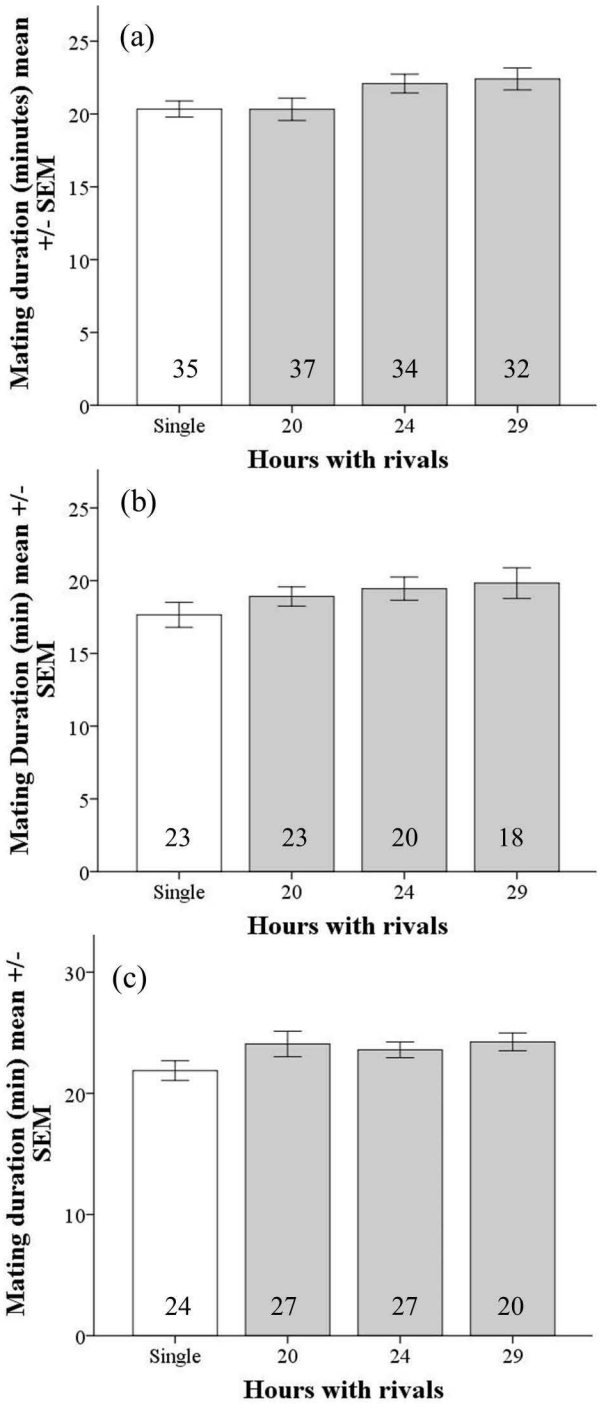

1
1a
1a
Exposure time
(0-72h)
Maintenance time
(0-48h)
2
2a
2a


(a)
**
**
Mating duration (min) mean +/- SEM
25
20
15
10
5
0
37
35
36
40
37
37
0
12
24
Time isolated (h)


(b)
***
Mating duration (min) mean +/- SEM
25
20
15
10
5
0
37
37
36
35
37
37
0
12
24
Time isolated (h)


(c)
*
*
Mating duration (min) mean +/- SEM
25
20
15
10
5
0
37
38
35
39
39
38
37
Single
0
1
3
6
9
12
Time isolated (h)


(a)
***
***
Mating duration (min) mean +/- SEM
25
20
15
10
5
0
40
34
40
33
36
39
0
12
24
Time isolated (h)
(b)
***
***
Mating duration (min) mean +/- SEM
25
20
15
10
5
0
26
26
27
31
33
25
0
12
24
Time isolated (h)
(c)
***
***
***
Mating duration (min) mean +/- SEM
25
20
15
10
5
0
36
32
40
33
37
29
0
12
24
Time isolated (h)


(a)
Mating duration (minutes) mean +/- SEM
25
20
15
10
5
0
35
37
34
32
Single
20
24
29
Hours with rivals
(b)
Mating Duration (min) mean +/- SEM
25
20
15
10
5
0
23
23
20
18
Single
20
24
29
Hours with rivals
(c)
Mating duration (min) mean +/- SEM
30
20
10
0
24
27
27
20
Single
20
24
29
Hours with rivals