

following nest exits. Every 2 to 3 days, nest position was changed in the horizontal plane on a 4 m long platform to make positional cues unreliable. The choice criterion for a returning bee was defined as the first colour landmark that the bee hovered in front of, or landed on. Thus, if the bee chose the training colour landmark, the response was considered correct. Data from all bees were pooled, and *G*-tests were used to determine whether choice distributions differed significantly from chance. With fewer than 20 choices, binomial tests were used.

In Test 1, bees chose darker and brighter yellows (Y1, Y2) as often as the training yellow (T) showing that they neglected intensity-related cues, while grey was chosen only once ($n = 58$, $G = 49.96$, $P > 0.001$; Figure 1E). In Test 2, bees discriminated the training yellow from both green (Ge1, Ge2) and grey (Gr1, Gr2; $n = 145$, $G = 350.6$, $P < 0.0001$; Figure 1E). Even when Test 2 was performed under the darkest conditions with the sun more than 12° below the horizon, and less than 10% of the moon visible (luminance of white stimuli between 10^{-3} and 10^{-5} cd m⁻²), the bees chose the training yellow (T) (Figure 1E, 'dark'). Bees from a third nest trained to a grey landmark, and tested with four yellow and green shades, chose grey in 29 of 31 trials ($G = 79.40$, $P < 0.0001$). When the training grey was substituted with a darker grey (Gr3 in Supplemental Figure S2), bees always chose the correct colour ($n = 8$, binomial test, $P < 0.01$).

Thus, the bees discriminated the landmarks using colour rather than intensity-related ('brightness') cues even at light intensities much lower than the human colour vision threshold. This is the first evidence of nocturnal colour vision in an animal with apposition compound eyes and the first demonstration of nocturnal colour vision in an animal's natural environment. *X. tranquebarica* is an active nocturnal forager on flowers that vary significantly in colour, and may rely on colour vision to distinguish them. Colour vision is useful for reliable object discrimination at night because crepuscular shifts in illumination colour [6] make 'brightness' unreliable: yellow (T) is brighter than grey (Gr1) in starlight but darker in twilight. The colours,

however, remain the same (Figures 1A, B and Supplemental Figure S2). Colour vision in starlight by bees with apposition eyes was unexpected — in dim light, diurnal bees apparently pool signals from the different spectral classes of photoreceptors to sacrifice colour vision in favour of an improved signal-to-noise ratio [2]. Exactly how nocturnal colour vision is achieved in apposition eyes remains to be elucidated experimentally, but increasing experimental and theoretical evidence suggests that neural summation mechanisms are likely to be necessary [7].

Supplemental Data

Supplemental data are available at [http://www.current-biology.com/supplemental/S0960-9822\(08\)01100-7](http://www.current-biology.com/supplemental/S0960-9822(08)01100-7).

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References

1. Peitsch, D., Fietz, A., Hertel, H., Souza J. D., Fix Ventura, D., and Menzel, R. (1992). The spectral input system of hymenopteran insects and their receptor-based colour vision. *J. Comp. Physiol. A* 170, 23–40.
2. Menzel, R. (1981). Achromatic vision in the honeybee at low light intensities. *J. Comp. Physiol. A* 147, 389–393.
3. Somanathan, H., Borges, R.M., Warrant, E.J., and Kelber, A. (2008). Visual ecology of Indian carpenter bees I: Light intensities and flight activity. *J. Comp. Physiol. A* 194, 97–107.
4. Kelber, A., Balkenius, A., and Warrant, E.J. (2002). Scotopic colour vision in nocturnal hawkmoths. *Nature* 419, 922–925.
5. Kelber, A., and Roth, L.S.V. (2006). Nocturnal colour vision — not as rare as we might think. *J. Exp. Biol.* 209, 781–788.
6. Johnsen, S., Kelber, A., Warrant, E.J., Sweeney, A.M., Widder, E.A., Lee, R.L., and Hernandez-Andres, J. (2006). Crepuscular and nocturnal illumination and its effect on colour perception by the nocturnal hawkmoth *Deilephila elpenor*. *J. Exp. Biol.* 209, 789–800.
7. Warrant, E.J. (2008) Seeing in the dark: vision and visual behaviour in nocturnal bees and wasps. *J. Exp. Biol.* 211, 1737–1746.

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Female presence is required for male sexual maturity in the nematode *Steinernema longicaudum*

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Although cheap compared to eggs, sperm are still costly to produce and may deteriorate if stored [1,2]. Therefore, selection should favour the ability of males to adjust the quantity or quality of sperm in response to fertilisation opportunities. The males of some species of insect, for instance, may adjust testis size, sperm number or ejaculate composition in response to the social environment [3]. Males of certain rodents, fish and insects increase sperm production in response to female presence [4–6]. However, males typically have at least some sperm always ready. Here, we show that in the insect-killing nematode *Steinernema longicaudum*, males that develop alone contain no sperm. Only after several hours with a female, but not another male, sperm are produced so that progeny can be sired.

We observed that male *Steinernema longicaudum* dissected from insect cadavers with single-worm infections contained no sperm in their seminal vesicles, while males that developed in insects containing many worms of both sexes all had seminal vesicles filled with sperm. This suggested that males require social experience in order to mature sexually. In a series of experiments carried out *in vitro*, in drops of insect blood, we investigated male sexual maturation (Supplemental data). Males that develop alone *in vitro*, as in insects, contained no sperm, but if they were subsequently paired with a female they contained sperm in the proximal region of the testis and in the seminal vesicle, and had a generally wider reproductive tract than naive males (Figure 1 and Supplemental data). When two males of *S. longicaudum* were incubated together for 24 hours, none of the 37 males examined contained sperm, showing that it is specifically female presence that stimulates sexual maturation, rather

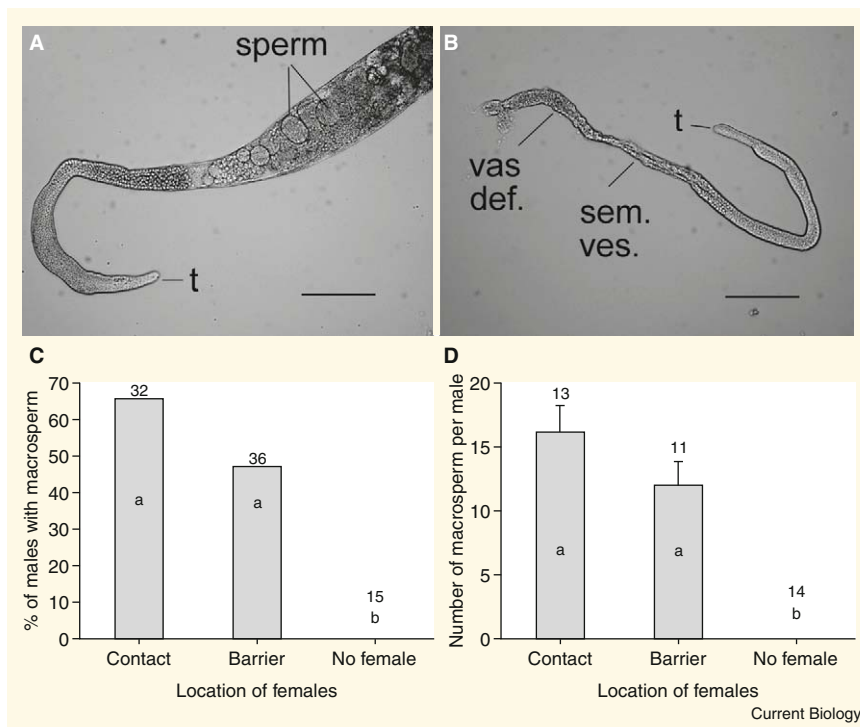


Figure 1. Effects of female presence on male maturation in the nematode *Steinernema longicaudum*.

(A) Mature male (24 h with female). (B) Naïve male (no female experience). The reproductive tract of *Steinernema* males is a simple tubular structure consisting of a single reflexed testis, a seminal vesicle (sem. ves; a dilated sperm storage region continuous with the testis) and a vas deferens (vas def.) leading to the cloaca. t = tip of testis. In the mature male, sperm are seen in the seminal vesicle; the vas deferens is not included in the photo. Scale bar, 100 μ m. The naïve tract is narrower than the matured one throughout its length, even at the distal region of the testis (see Table S1). (C,D) Proportion (%) of males with macrosperm (C) and number (mean \pm SE) of macrosperm (D) compared in whole-worm mounts of males that had been either in direct contact with females (Contact), separated from females by a permeable barrier (8 micron pore size) (Barrier), or without females (Control). Values within a panel followed by the same letters do not differ significantly. *n* indicates number of worms examined microscopically. Proportion of males with macrosperm compared by χ^2 (with *p*-value adjustment using Bonferroni-Holm step down approach): Contact versus Control: $\chi^2 = 17.80$, $p < 0.0001$; Barrier versus Control: $\chi^2 = 10.63$, $p = 0.001$; Contact versus Barrier $\chi^2 = 2.33$, $p = 0.1271$. Number of macrosperm per male compared using Bonferroni-Holm test.

than a more general social signal. The presence of sperm in the seminal vesicle of paired males was strongly associated with mating success ($\chi^2 = 40.4$, $p < 0.0001$; Supplemental data). It took up to 48 h spent with a female before all males had sperm and all had mated successfully. However, once mature, a male can inseminate several females in a short period of time (at least three females in four hours; Supplemental data). In contrast to males, 50% of naïve solitary-reared females ($n = 28$) mated within one hour when placed with a sexually mature male, indicating that a high proportion of females are receptive without prior social contact.

Males separated from females by a permeable barrier still produced

sperm (Figure 1), suggesting that *S. longicaudum* females release chemicals that stimulate sexual maturity in male worms. Many species of nematode produce sex pheromones that attract members of the opposite sex [7]. A stimulatory effect (as opposed to attraction) of nematode sex pheromones has not previously been reported, though social effects on gametogenesis are mediated by chemicals, including pheromones in other animal taxa [4,8,9].

Why should a male *S. longicaudum* delay spermatogenesis? A solitary *Steinernema* male or female can survive in an insect cadaver for up to 6 weeks. Such a solitary worm cannot suddenly encounter another reproductively mature individual, as

only juveniles invade insects. Sperm that are produced too long in advance of fertilisation may become less viable, leading to the evolution of traits to avoid the use of aged sperm [2]. In addition, individuals must balance the energy allocation required for basic somatic needs and that required for reproductive effort [10]. While sperm production has traditionally been regarded as cheap, there is abundant evidence that the resources needed to produce and package sperm can be costly. We estimate that the reproductive system of a sexually developed male *S. longicaudum* (including the testis and sperm-filled seminal vesicle) represents 30–40% of the body volume. We assume that the delay in sexual development in male *S. longicaudum* until females are available for reproduction is an adaptive behaviour, but the precise benefits remain to be elucidated.

Supplemental Data

Supplemental data including experimental procedures and discussion are available at [http://www.current-biology.com/supplemental/S0960-9822\(08\)01260-8](http://www.current-biology.com/supplemental/S0960-9822(08)01260-8).

References

- Olsson, M., Madsen, T., and Shine, R. (1997). Is sperm really so cheap? Costs of reproduction in male adders, *Vipera berus*. *Proc. R. Soc. Lond. B* 264, 455–459.
- Reinhardt, K. (2007). Evolutionary consequences of sperm cell aging. *Q. Rev. Biol.* 82, 375–393.
- Gage, M.J.G. (1995). Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. *Proc. R. Soc. Lond. B* 261, 25–30.
- Stacey, N. (2003). Hormones, pheromones and reproductive behavior. *Fish Physiol. Biochem.* 28, 229–235.
- Demas, G.E., and Nelson, R.J. (1998). Social, but not photoperiodic, influences on reproductive function in male *Peromyscus aztecus*. *Biol. Reprod.* 58, 385–389.
- Bjork, A., Dallal, R., and Pitnick, S. (2007). Adaptive modulation of sperm production rate in *Drosophila bifurca*, a species with giant sperm. *Biol. Lett.* 3, 517–519.
- Mackinnon, B.M. (1987). Sex attractants in nematodes. *Parasitol. Today* 3, 156–158.
- Rekwot, P.I., Ogwu, D., Oyedipe, E.O., and Sekoni, V.O. (2001). The role of pheromones and biostimulation in animal reproduction. *Anim. Reprod. Sci.* 65, 157–170.
- Pereira, R., Teal, P.E.A., Sivinski, J., and Dueben, B.D. (2006). Influence of male presence on sexual maturation in female Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae). *J. Insect Behav.* 19, 31–43.
- Bell, G., and Koufopanou, V. (1986). The cost of reproduction. In *Oxford Surveys in Evolutionary Biology*, Vol. 3, R. Dawkins and M. Ridley, eds. (Oxford: Oxford University Press), pp. 83–131.

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