looking at short-term effects have shown, in agreement with the finding of Collins, Boddington *et al.*[3], that VNS causes a widely distributed increase of neural activation[13,14] (Figure 1A,B), those examining the long-term impacts have reported a progressive pattern of physiological habituation leading to a gradual deactivation in many prefrontal and limbic structures (Figure 1C)[19,20]. In other words, over time, brain responses to VNS become more complex, moving from a general increase in activation to a more intricate pattern of neural activation/deactivation states. It is tempting to speculate that this evolution relies, in part, on the mixed (bidirectional) nature of the vagus nerve's anatomical organisation[14]. Indeed, the reactivation of interoceptive afferent signals that modulate neural activity through closed-loop interactions between the body and the brain is likely to take time and require the design of long-term longitudinal studies to be identified.

To summarize, the study by Collins, Boddington *et al.*[3] provides stimulating results that will probably fuel future research into how VNS can have a positive impact on such a wide range of neurological, medical and psychiatric conditions.

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Sexual selection: Large sex combs signal male triumph in sperm competition

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A new study using artificial selection reveals that the size of the sex comb on the legs of male flies is genetically correlated with their fertility success under conditions of sperm competition.

As spring hits the animal world, out come gaudy lures, whirling dances, potent smells, shrieks and yelps as males — and sometimes females — attempt to attract liaisons, brief or prolonged, with the opposite sex. Males are happy to trade investment in sexual displays for gains in reproduction. Females compare and assess displays to gain benefits for themselves and their

offspring. Where direct gains (food, nests, territory) are absent, genetic endowment is usually invoked as the profit of the female gaze, even though it has proven hard to establish beyond reasonable doubt. A plausible alternative is that ornaments directly indicate male fertility, his ability to sire a large brood, triumph in competition against other males' ejaculates and pass on potent sperm production to sons in the next generation. Unfortunately, this idea, branded the "phenotype-linked fertility hypothesis"[1], remains contentious for a simple reason: the more desirable a male, the more he mates and the less he has to invest per mating[2]. Fertility benefits aren't fixed: they inevitably are diluted, especially among more attractive, multiply mating males[3–5]. A recent study in *Current Biology* by Michal Polak and colleagues[6] challenges this pessimistic view with new experimental evidence on the sex combs of the fly *Drosophila bipectinata* (Figure 1).

Males use sex combs, teeth-like bristles on their front legs, to gently grasp and caress their partners' abdomens in pre-copulatory foreplay. Females respond favourably and proceed to copulation when stimulated by well-endowed males sporting larger combs. To uncover what advantages females gain from their mate choice, artificial selection was applied to comb size on *D. bipectinata* flies collected from the field. Comb size showed high heritability, with average tooth number going from a baseline of 13 to 16 in the high and 10 in the low selected lines (anaesthetize 100 males, and repeatedly pick the 30% highest or lowest). After 11 generations of selection, males from the lines were tested in a classic 'P_2' sperm competition experiment. Standard females were mated first to an irradiated stock male and then to the focal test male. Irradiation was set to a sub-lethal dose which leaves sperm able to fertilise, but embryos die due to lethal mutations. The proportion of eggs producing offspring after the second mating (P_2) reflects the competitive fertilizing success of the second test male. In males from the lines selected for high comb number, P_2 was strongly elevated.

Figure 1. Male sexual ornament associations with fertility.
During sexual engagement, male *Drosophila bipectinata* vibrate the sex combs on their tibia across the female's abdomen (top left; image: Michal Polak). Artificial selection[6] reveals that the size of the sex comb is genetically correlated with a male's competitive fertilization success against a prior male's ejaculate (P_2). A similar positive correlation between sexual ornaments and sperm traits associated with fertility has been found in the zebra finch, *Taeniopygia guttata*[20] (center right). But, contrary to this, *Poecilia reticulata* guppies (bottom right; image: Per Harald Olsen/Wkicommons) with more showy colours have slower swimming sperm[14], males of the Australian cricket, *Teleogryllus oceanicus* (bottom left; image: Nathan W. Bailey (CC BY 4.0)), which genetically produce longer song trills, have fewer viable sperm in their ejaculates[15] and domesticated chickens (top right; image: Francesco Veronesi (CC BY-SA 2.0)) selected for larger head combs end up with reduced testis size[16]. This mix of positive and negative correlations reveals that investment trade-offs are likely to place limits on sexual ornament size as an indicator of a male's capability to succeed in sperm competition or to enhance female fertility.

Artificial selection thus beautifully uncovered an underlying genetic correlation between comb size and male fertilizing success. The obvious mechanistic reason for this association is that larger combs cause greater female arousal and preferential sperm use, so called cryptic female choice. However, this explanation was cleverly dismissed by Polak and colleagues[6]. They used a fine scale laser to ablate individual teeth from the sex combs of high-line males, bringing the number down to low-line levels. To their surprise, this phenotypic manipulation had no impact on competitive fertilization success. Ablated high-line
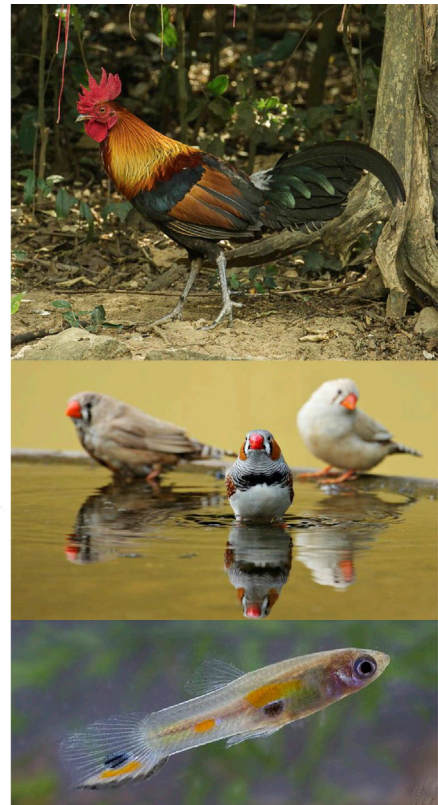
males achieved elevated P_2, no different from their non-ablated relatives. What can be baldly stated is that the experimentally induced difference in sex comb size simply does not explain preferential fertilization success.

A number of other candidate traits that might explain the greater fertilization success of high-line males were ruled out by Polak and colleagues[6]. Body size is often correlated with fertility, as it was also here. But as the artificial selection regime controlled for size, there was no difference between lines. Increased male mating rate, testis size and accessory gland size all seem good candidates for the higher fertilization

success, but none of these were greater in high-line males. More promising, a comparison of mRNA transcripts identified three ejaculate proteins transferred from the male accessory glands to the female at mating that were upregulated in high lines. Fortunately, these proteins have been previously studied in *Drosophila melanogaster.* One of these proteins is the well-known sex peptide, which has a range of effects on female sexual behaviour including suppressing their propensity to remate and enhancing male siring success in sperm competition[7,8]. The other two are serine proteases that enhance male fertility[9]. Exactly how these ejaculate components work in *D. pectinata* is not known. They appear to increase high-line sperm viability when extracted from the female's ventral receptacle, a cassette-like structure where sperm are individually stored before release into the oviduct. To survive in this location, male ejaculates contain a diversity of proteins that confer resistance to female spermicidal secretions[10]. This might explain why upregulation of these proteins is associated with increased high-line sperm competitiveness.

A key question posed by the results is the reason for the unravelled genetic association between a sexual ornament and fertilizing capacity. Polak and colleagues[6] suggest that attractive males more often encounter intensive sperm competition. This should lead to selection shaping their ejaculates to cope with competitive environments, much more so than the ejaculates of undesirable males. Sperm competition theory certainly supports the idea that competition favours larger or more potent ejaculates with greater 'fertilizing power' when competition increases[11]. But this prediction is based on population-level comparisons, where there are particular male types, for example territory-holders and satellites, which differ radically in their opportunities to mate[12]. This selective logic might apply in *D. pectinata* if males adorned with large combs excite previously mated females to re-mate earlier in their sexual cycle, well before the female has exhausted sperm stored from previous males. Then, ejaculates

of large-comb males will typically encounter greater numbers of rival sperm and will profit from greater investment in costly seminal proteins to overcome them. However, it remains to be shown whether females are indeed more willing to remate when encountering highly ornamented males as suggested. This explanation predicts that the greater 'offensive' capabilities (second male success) of attractive males does not carry over to 'defence' against rival sperm (ability to withstand competition from subsequent ejaculates) as the 'strength' of subsequent male ejaculates is not predictably linked to the current male's attractiveness. This prediction is yet to be examined.

There are more generic concerns about the notion that male sexual ornaments are associated with greater fertility[13]. Higher investment in attractiveness is never for free, often being traded-off against reduced fertility (Figure 1). For example, there is a negative genetic correlation between sperm quality and colour ornaments in the guppy[14] (*Poecilia reticulata*) and with courtship song in crickets[15] (*Teleogryllus oceanicus*). Moreover, directional selection for increased comb size (in this case, the red fleshy crest on the male's head) in the domestic chicken (*Gallus domesticus)* generated a correlated reallocation of resources away from testes[16]. Worse follows, as the selective rationale of having larger sexual ornaments is to gain more matings. So given a limited budget, attractive males inevitably must divide their seminal resources into multiple, smaller ejaculates with likely reductions in their competitive fertility[2,17].

These considerations make the explanation of Polak and colleagues[6] based on enhanced sperm competition harder to swallow. A way out of this conundrum is that well-adorned males are simply of higher quality. It is an old and well-established idea that exaggerated sexual ornaments are condition-dependent handicap traits that signal male genetic and phenotypic quality[18]. High-quality males with larger sexual ornaments expect greater female interest and so they should suitably increase investment in reproductive traits, such as sperm or ejaculate

proteins. Whether the imperative to service extra females results in more or less investment per ejaculate with sexual ornament size then depends on all the spice of life-history, such as the propensity of females to mate with several males (polyandry), her mate preference, male costs of sexual trait exaggeration, as well as the degree of sperm competition.

Ultimately, females also need to reap benefits from preferentially mating with ornamented males and will thus evolve to have reproductive tracts structured to favour particular ejaculate properties[19]. What Polak and colleagues[6] have convincingly demonstrated is a positive genetic covariance between a sexual ornament and competitive fertilization success. These traits should be inherited, allowing dad's success to endow his sons. If the condition-dependent hypothesis holds up, there are also associated 'good genes' for other aspects of male fitness to be passed on as well. We need to know a lot more about this before the whys and wherefores of *D. bipectinata* sex combs come fully into focus.

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Social insects: Stochastic switches and behavioral maturation in ants

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Changes in behavior with age are widespread in social insects. Lifetime behavioral tracking of individual ants shows these changes are fast and stereotyped, yet stochastically timed. The finding may help shed light on the mechanisms of behavioral maturation and their role in division of labor in social insects.

Social insects are widely heralded as a pinnacle of behavioral evolution: they make intelligent collective choices[1] and efficiently divide group labor between hundreds, thousands or even millions of individuals[2], all in the absence of centralized control[3]. Despite more than a century of study, however, many aspects of group coordination in social insects remain mysterious, in part because of the challenges inherent in studying the behavior of so many individual animals over extended colony life cycles. In a paper in this issue of *Current Biology*, Thomas Richardson, Laurent Keller and colleagues[4] take advantage of emerging tools for precisely tracking individual workers within ant colonies and use this approach to shed new light onto a canonical example of division of labor in

social insects: predictable changes in behavior with age.

Division of labor — the specialization of individual workers on particular tasks — is considered a key adaptation in social insect evolution that can improve task performance and efficiency. Absent central control, however, dividing labor among workers poses a challenge with potentially disastrous consequences; too many foragers could lead to a stockpile of wasted food, while too few defending the nest could leave a colony susceptible to predation.

Some well-known examples of division of labor involve specialization between morphologically distinct groups of workers, such as the overgrown soldiers of leaf cutter ant *Atta sexdens* that specialize on defense and protection[5].

However, such morphological specialization is actually relatively rare[3]. Rather, the most common pattern of division of labor is wonderfully simple: tasks change predictably with an individual's age. Young members of the colony perform one set of tasks, gradually shifting to other tasks as they age. In honey bees, for example, newly emerged workers focus on nursing — cleaning cells, feeding and capping developing brood — for several days, and then perform other within-nest tasks — grooming and feeding nestmates, ventilating the hive — before eventually transitioning to foraging outside the nest after roughly two weeks[6]. This behavioral progression is correlated with spatial shifts; workers spend most of their time near the nest center initially, then