would need to be at least twice that size. And making coral reef marine protected areas nearly 20 times that size would still not be enough to cover 100% of that species' activity space.

To be fair to the environmental advocates who work hard on the important task of creating marine protected areas, it should be noted that not all marine protected areas around coral reefs are designed to protect sharks. Therefore, being too small to protect sharks does not necessarily represent a failure. However, many marine protected areas do indeed state shark protection as a goal [8]. Additionally, many marine and terrestrial protected areas aim to protect not a species' entire activity space, but a piece of particularly significant habitat associated with mating or migration — if we cannot keep a species totally isolated from all threats, we can at least try to protect it when it is most vulnerable. Indeed, Dwyer and colleagues [6] did find a benefit to sharks, in the form of slightly reduced mortality from fisheries, even from marine protected areas that are far too small to protect these animals' entire activity space. But there is no doubt from their analysis that such a benefit pales in comparison to the benefits offered by a larger protected area.

Additional important concerns about the effectiveness of marine protected areas as a tool for shark conservation have been raised this year. A practical guide for conservation practitioners [9] notes that some of the most common reasons why shark-focused marine protected areas fail to protect sharks fall into the realm of social science, not biology or fisheries science; for instance, failure to understand the needs of stakeholders and failure to get community members to support the marine protected area lead to likely failure of the marine protected area. The study of Dwyer and colleagues [6] is an important reminder that sometimes we do still need some fairly basic biology and behavior data in order to maximize the chances of a protected areas success.

Marine protected areas can work ocean conservation wonders in some cases, and with calls from the IUCN to fully protect 30% of the ocean by 2030, they are a policy solution that is undoubtedly here to stay. However, marine protected areas created without the best available scientific data run the risk of failing to accomplish their important goals. Studies like that of Dwyer and colleagues [6] will play a critical role in making sure that marine protected areas can do the most good possible.

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Mate Choice: Should I Mate or Should I Go?

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A mature virgin female fruit fly will initially resist copulation, while she assesses the desirability of her suitor. A new study identifies a neural circuit that controls rejection and shows how it changes from rejection to acceptance and copulation.

The courtship ritual of a male fruit fly (*Drosophila melanogaster*) consists of choreographed steps of behaviors. He will tap, follow, lick and produce a sophisticated courtship song by vibrating a wing. If he is successful, copulation occurs [1]. In contrast, the female's behavior appears to mostly consist of running away and kicking, followed by eventual slowdown and copulation. It would be a mistake to underestimate her role, however, because *Drosophila* females are the ones to decide whether copulation occurs or not [2–4]. His job is to convince her that he is

from the correct species and a highly desirable mate. The female then faces a critical decision: to mate or not to mate? The survival of her genes depends on her prudent decision. His pheromone profile and his courtship song, together with other cues, may identify him as a desirable mating partner.

But the courted female fly has other important things to consider. Is she a mature virgin, ready to mate, or is she an immature virgin? Or has she already mated? During mating, males transfer accessory gland proteins together with the sperm, most prominently the so-called sex peptide. These peptides cause a profound behavioral change in the female. She will start to vigorously reject other courting males by running away, kicking and extrusion of the ovipositor (the appendix through which she lays her eggs), and not allow further copulations for over a week [5,6]. Immature virgin females (less than 48 hours old) will resist courtship and attempted copulation just as vigorously by running away and kicking [7]. Physiologically, the most obvious difference between a young and a mature virgin female is the presence of mature eggs in the ovaries of the latter. But little is known about the molecular processes that underlie behavioral 'maturation', the transition from unreceptive virgin to a virgin ready to mate.

Even a mature virgin female needs time to assess the courting male. She will initially reject him by running and kicking, before rejection gives way to acceptance and copulation [2]. She will signal her acceptance by slowing down, allowing the male to come close, and will then open her wings and her vaginal plates to enable copulation. The pre-mating rejection response is evolutionarily observed in many species, but its molecular and neuronal basis is largely unknown. It is an attractive model for studying the neural processes of decision making. In this issue of *Current Biology*, Ishimoto and Kamikouchi [8] report the identification of a novel neuronal circuit that underlies the pre-mating rejection response in *Drosophila*, and propose a feedforward mechanism for its switch to acceptance.

Ishimoto and Kamikouchi [8] paired individual mature virgin females with a mature wild-type male in a small circular chamber and recorded their behavior using a semi-automated assay. They scored latency (time to copulation) and copulation rates at different time points during the 50-minute observation period. To identify relevant neurons and their specific role in the control of this behavior, they genetically manipulated candidate neurons and studied the effect of either silencing or activating them. The silencing of neurons that are responsible for rejection is expected to make the female less resistant to copulation, leading to an increase in copulation rate and a shortening of latency. Conversely, overactivation of these neurons would increase rejection and latency.

To manipulate neurons, Ishimoto and Kamikouchi [8] used the Gal4/UAS genetic system, which allows the expression of a protein of choice in a cell of choice [9]. In this system, transgenic flies are produced that carry several transgenes. A tissue-specifically expressed Gal4 transcription factor binds to the upstream activating sequence (UAS) upstream of a second transgene, and controls expression of an effector of choice — a reporter protein, for example, or ion channels that can activate or silence neurons. This results in the cell-specific expression of the effector. Many cell-specific Gal4 transgenes are available that allow the interrogation of specific neurons. As courtship is an adult behavior, it is desirable to manipulate cells only in adult flies in order to observe acute neuronal processes. This can be achieved with either a temperature-sensitive effector or by the addition of a temperature-sensitive Gal4 inhibitor ($Gal80^{ts}$) to the system [10].

Two brain clusters in the female brain have been identified that process information about a male-specific pheromone and male courtship song [11,12]. They are located in the superior medial protocerebrum (SMP), a region that contains dopaminergic neurons, but no direct neuronal contacts have been demonstrated yet. A role for dopamine in the modulation of female receptivity has been shown before [13], but how exactly this relates to rejection/acceptance behavior is unknown. Ishimoto and Kamikouchi [8] chose fly lines that express Gal4 in subsets of dopaminergic SMP neurons and examined what happens when these neurons are silenced. They found that silencing of a particular set of neurons decreased rejection, as evidenced by faster mating and higher overall copulation rate, suggesting a role for these neurons in rejection.

Ishimoto and Kamikouchi [8] next found that these dopaminergic neurons project to neurons in the ellipsoid body (EB) of the fly brain. The EB contains layers of ring neurons with diverse functions and is part of the central complex, a well-studied insect higher-order brain center [14]. The authors discovered that two subsets of EB neurons, R4d and R2/R4m, form a functional circuit that controls initial rejection and the subsequent transition to copulation (Figure 1). Both sets of neurons act in response to dopamine, albeit through different receptor subtypes. Cholinergic signaling from R4d neurons was found to be responsible for rejection behavior. When R4d neurons are silenced, the copulation rate increases while latency decreases. Interestingly, silencing of the R2/R4m neurons has the opposite effect: copulation is delayed. It thus appears that the two kinds of neurons have opposite roles in the rejection circuit. Could their dynamic interaction and relative strength determine the balance between rejection and acceptance?

Ishimoto and Kamikouchi [8] found that R2/R4m make direct synaptic contact with R4d, but not *vice versa*. Overall, the results from their experiments suggest that the decision from rejection to acceptance progresses in at least two steps: Initially, cholinergic signaling from R4d neurons leads to rejection. GABA

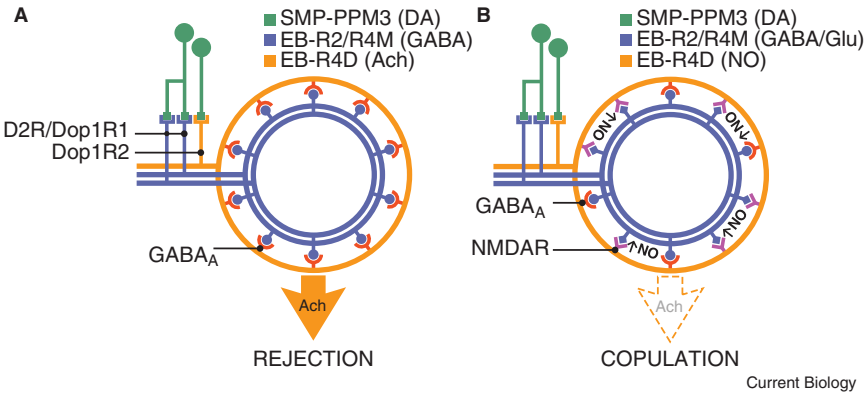

Figure 1. A neuronal feedback loop controls the decision to switch from pre-mating rejection to copulation in mature virgin females.
Two different subsets of ring neurons in the ellipsoid body (R2/R4m and R4d) receive input from dopaminergic neurons through different sets of dopamine receptors (D2R/Dop1R1 and Dop2R2, respectively). (A) Cholinergic R4d signaling mediates rejection. R2/R4m neurons interact with R4d through GABA and a $GABA_A$ receptor located on R4d. (B) This initial interaction leads to NO production in R4d that feeds back and enhances R2/R4m signaling. Enhanced GABA and glutamate signaling now act through $GABA_A$ and NMDA receptors to silence R4d and to abolish rejection. This enables copulation. Picture by Mike de la Flor.

signaling from R2/R4m to R4d weakens the rejection response but does not abolish it (Figure 1A). But this first GABA signal triggers synthesis of the diffusible second messenger nitric oxide (NO) in R4d; NO then diffuses back to R2/R4m and increases the strength of R2/R4m signaling. In the second step (Figure 2B), the increased signal coming from R2/R4m activates NMDA receptors in R4d that now also respond to the glutamate that originates at R2/R4m. Together the transmitter systems silence the R4d-mediated rejection response and enable the transition to acceptance and copulation.

The identification of these circuits by Ishimoto and Kamikouchi [8] is an important step forward in understanding how mating decisions in mature virgin females are made. One of the attractive features of this multi-component circuit is its flexibility. This starts at the very top, where both sets of neurons receive dopaminergic input, but process it through different receptors, allowing for different downstream processing. Acceptance can be delayed or accelerated, or copulation denied altogether. The dynamics of the response from rejection to acceptance will likely depend on the female's assessment of the quality of the courting male. How females integrate this information into the circuit will be a very interesting question to follow up on. It is possible that the identified neurons are not the only decision-making input into the circuit. When the described circuit neurons were manipulated, the copulation rates dropped dramatically in some instances, but they did not drop to zero, and low numbers of copulations still took place. This is in stark contrast to what is observed in immature virgin females, where despite vigorous courting by males, copulations are almost never observed. It will be interesting to explore how the immature rejection pathway is related to the circuit described here, and what "maturation" means in this context. Parallel pathways may exist for mating decisions depending on the state of the female. The authors found that manipulation of the R4d neurons did not change the strong post-mating rejection behavior of already mated females. In the question of whether to mate or to run there appears to be no one right answer.

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