Similarly, in the Murayama *et al.* study a key question involves the assay *per se*, which relies on the core assumption that the admittedly very stable interaction observed between cohesin and DNA is topological, i.e. that the observed binding reflects real DNA entrapment inside cohesin rings. Previous experiments employing chemical crosslinking in budding yeast cells have proven that single cohesin rings are able to trap small circular yeast sister chromosomes [2,3]; all experimental evidence corroborates a model whereby this entrapment indeed mediates sister chromosome cohesion [17]. This could be definitively proven in a cell-free system if the assay was supplemented with chemically cross-linked cohesin rings, as done previously inside yeast cells.

Despite having a slow start, the enzymology of Smc–kleisins is finally coming of age. Condensin and a handful of other factors can reconstitute entire chromosomes [18] without even the help of nucleosomes [19]. Loop extrusion is most likely the way in which all Smc–kleisins work DNA into loops [20]. Nevertheless, condensin and cohesin appear to have evolved to do different things at different moments in the cell cycle. Their core reactions might prove very similar, but the complete mechanisms are probably substantially different, reflecting their very different tasks in shaping chromosomes.

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Sex: The End Is All You Need

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Sex is rewarding. Out of the many steps needed for successful mating, from courtship through copulation, the ultimate ejaculatory step in male fruit flies has profound rewarding properties.

It almost goes without saying that sex is rewarding. Sex is needed for the survival of species, and the sex–reward relationship is well established in mammals [1]. That sex is rewarding in insects too, therefore, should come as no surprise [2]. What is less clear is whether a single component of courtship and copulation might be rewarding. In a recent paper in *Current Biology*, Shir Zer-Krispil, Galit Shohat-Ophir and colleagues [3] report on a neural circuit that reveals that the ultimate ejaculatory step in the *Drosophila melanogaster* courtship ritual

is rewarding. It turns out that stimulation of sets of neurons that produce either the neuropeptide corazonin or its receptor are sufficient for triggering ejaculation, and that this is rewarding to the fly.

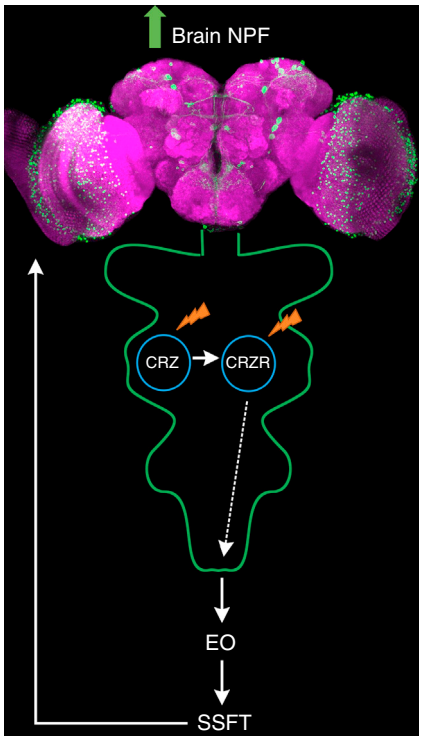
For male *Drosophila, c*ourtship and copulation follow stereotypical steps. Male flies will orient toward a female, beat a wing in a species-specific fashion, tap a female with a foreleg, sometimes touch her abdomen with his proboscis and attempt copulation [4,5]. If courtship of a receptive female is successful, most males will start copulating in about 6 or 7 minutes. Copulation lasts for a little over 20 minutes. If courtship is unsuccessful then flies will court a second female with less vigor. After rejection, if a male is given a choice he will eat more food laced with ethanol than without [2]. Moreover, expression levels of Neuropeptide F (NPF), the fly orthologue of vertebrate Neuropeptide Y (NPY), is low [2]. NPF and NPY have both been implicated in rewarding mechanisms [6,7]. This suggests that courtship and copulation are rewarding to the male fly and that rejection of courtship leads flies to seeking alternative rewards.

Clues about which parts of the nervous system regulate courtship have come from anatomical and functional characterizations. Some neurons of the abdominal ganglion, a part of the fly' central nervous system that is outside of the head, are specific to male flies. These male-specific neurons include some of the corazonin and corazonin-receptor neurons. The corazonin-receptor neurons also likely use serotonin as a neurotransmitter. Reduced function of the serotonergic neurons has an adverse effect on copulation [8]. Moreover, decrease in the activity of the corazonin-containing neurons, upstream of the serotonin neurons, leads to prolonged copulation [9]. Therefore, the male-specific corazonin and corazonin-receptor neurons were targeted in the new study of Zer-Krispil and colleagues [3] to see if extrinsic activation might be rewarding to male flies.

To get a first clue of whether the male-specific corazonin and corazonin-receptor neurons are rewarding, Zer-Krispil and colleagues [3] expressed a light-sensitive Cs-Chrimson ion channel [10] in corazonin neurons and put flies in a behavioral assay where part of a chamber would be illuminated by red light (a type of light that is invisible to flies) [3]. The idea is if turning on Cs-Chrimson with red light, and thus activating a set of neurons, is pleasant to the flies, they would spend more time in the lit part of the chamber. The authors observed that once the male flies went into the 'red-light zone', so to say, the flies ejaculated (Figure 1). Flies also spent more time in the lit part of the chamber than in control areas or situations.

Importantly, to test whether the corazonin and corazonin-receptor neurons are indeed rewarding, Zer-Krispil and colleagues [3] used two approaches: classical olfactory conditioning and measurement of brain NPF levels. In the first approach, they trained flies to associate an odor with optogenetic red light stimulation of the corazonin neurons. The flies were then allowed to choose between the odor associated with activation and another odor not associated with activation. Male flies displayed a strong preference for the odor associated with activation of the corazonin neurons and ejaculation for several minutes after conditioning. This shows that corazonin neuron activation is rewarding. Previous studies have shown that mating increases NPF levels in flies [2]. Zer-Krispil and colleagues [3] have quantified the brain NPF transcript levels in response to repeated activation of male-specific corazonin neurons. Flies with activated corazonin neurons had significantly higher NPF transcript levels compared to flies in which corazonin neurons were inactive, experimental controls, and female flies. The corazonin-receptor neurons are also part of the neural circuit involving reward, and activation of corazonin-receptor neurons is also rewarding to male flies.

Successful mating not only increases NPF levels but also reduces the propensity of flies to consume ethanol-laced food [2]. Having established that activation of either corazonin or corazonin-receptor neurons is rewarding, the authors proceeded to test whether this is sufficient to reduce ethanol intake, itself a rewarding experience, in male flies [11]. Consistent with previous studies, repeated activation of corazonin neurons



Current Biology

Figure 1. A neural circuit stimulates ejaculation and links to pleasure centers in the *Drosophila* brain.
Activation of corazonin neurons (CRZ) and corazonin receptor neurons (CRZR) leads to sperm and seminal fluid transfer (SSFT) and increased NPF levels in *Drosophila* male brains. NPF neurons are labeled with GFP [12]. Processing downstream of CRZR at the level of ejaculatory organs (EO) might also be involved in increasing brain NPF levels. Activation of the SSFT circuit is rewarding.

in sexually inexperienced males reduced the preference of flies to consume ethanol-containing food. Control flies, however, consumed relatively more ethanol-laced food. Thus, the rewarding property of ejaculation applies to another rewarded behavior.

Many animals follow elaborate courtship rituals to earn mating rights with potential partners. However, it was unknown which, if any, of those individual steps might have the rewarding value. Through activation of corazonin and corazonin-receptor neurons, Zer-Krispil and colleagues [3] revealed a single stage, ejaculation, the final step in mating, as the rewarding experience in flies. They discovered that brain NPF levels went up with activation of these neurons. Moreover, virgin male flies with activated

corazonin neurons had the same propensity towards ethanol as mated wild-type males, suggesting that the rewarding property of ejaculation influences a second pleasure seeking behavior.

Though extensively studied, the neural circuitry underlying mating and reward in flies still offers room for discovery. What is the rewarding value of corazonin and corazonin-receptor neurons in the natural context of courtship and mating? Are other parts of the courtship ritual rewarding? How do different reward pathways intersect with mating pathways? Is the rewarding value of induced ejaculation synonymous with the whole routine of courtship and mating? Finally, are there rewarding neural circuits in courtship/copulation for female flies? The study by Zer-Krispil and colleagues [3] has provided a critical piece of the puzzle in the broader goal of understanding the complete neural circuitry involved in the reward of sex.

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Respiration: Life Without Complex I

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Eukaryotic life has developed a fascinating and highly optimized system for energy transduction: the mitochondrial respiratory chain. Typically composed of five core protein complexes, we now learn from two studies that plant hemi-parasites of the type *Viscum* cope without Complex I, the entry point of the classical respiratory system.

At the onset of eukaryotic evolution, a primitive cell merged with an α-proteobacterium, which in time evolved into a mitochondrion specialized in converting the energy of redox equivalents into ATP [1]. Classically, five transmembrane protein complexes in the inner mitochondrial membrane are involved in this process: Complex I, an NADH:ubiquinone oxidoreductase; Complex II, a succinate dehydrogenase; Complex III, a ubiquinol:cytochrome *c* oxidoreductase; Complex IV, the cytochrome *c* oxidase; and Complex V, the ATP synthase. The electron transport chain is branched: Complex I and Complex II deliver electrons via the mobile electron-carrier ubiquinone to Complex III. In plants, alternative NAD(P)H dehydrogenases exist, which re-oxidize NAD(P)H either at the matrix side (ND_{int}) or at the intermembrane space (ND_{ext}) [2]. In addition, Complex IV can be bypassed by an alternative oxidase, AOX (Figure 1, upper panel) [3]. These additional respiratory complexes enable significant plasticity in the plant respiratory chain. Moreover, Complex I, Complex III and Complex IV can assemble into supercomplexes of different compositions and stoichiometries — likely also an adaptive mechanism [4–6].

Complex I is the main entrance gate for NADH electrons into the electron chain. It is one of the most sophisticated complexes, comprised of 14 central subunits and up to an additional 30–34 accessory subunits [7]. This complexity